Metabolomics

January 8, 2021

[127]:
```
import pandas as pd
import matplotlib.pyplot as plt
import matplotlib.patches as mpatches
from sklearn.cluster import KMeans
import seaborn as sns
import numpy as np
from scipy.stats import zscore,ttest_ind,norm, hypergeom
from statsmodels.stats.multitest import multipletests
from sklearn.decomposition import PCA
%matplotlib inline

sns.set(context='talk',style='ticks',font_scale=1,rc ={'axes.spines.right':␣
↪False,'axes.spines.top': False,'pdf.fonttype':42,'ps.fonttype':
↪42},palette='colorblind')
```

1 Figure 3 C: Targeted amino acid quantification

1.1 Define the Q-test for identification of outliers in the AA Quantification

[2]:
```
q90 = [0.941, 0.765, 0.642, 0.56, 0.507, 0.468, 0.437,
       0.412, 0.392, 0.376, 0.361, 0.349, 0.338, 0.329,
       0.32, 0.313, 0.306, 0.3, 0.295, 0.29, 0.285, 0.281,
       0.277, 0.273, 0.269, 0.266, 0.263, 0.26
      ]

q95 = [0.97, 0.829, 0.71, 0.625, 0.568, 0.526, 0.493, 0.466,
       0.444, 0.426, 0.41, 0.396, 0.384, 0.374, 0.365, 0.356,
       0.349, 0.342, 0.337, 0.331, 0.326, 0.321, 0.317, 0.312,
       0.308, 0.305, 0.301, 0.29
      ]

q99 = [0.994, 0.926, 0.821, 0.74, 0.68, 0.634, 0.598, 0.568,
       0.542, 0.522, 0.503, 0.488, 0.475, 0.463, 0.452, 0.442,
       0.433, 0.425, 0.418, 0.411, 0.404, 0.399, 0.393, 0.388,
       0.384, 0.38, 0.376, 0.372
       ]
```

```
Q90 = {n:q for n,q in zip(range(3,len(q90)+1), q90)}
Q95 = {n:q for n,q in zip(range(3,len(q95)+1), q95)}
Q99 = {n:q for n,q in zip(range(3,len(q99)+1), q99)}


def dixon_test(data, left=True, right=True, q_dict=Q95):
    """
    Keyword arguments:
        data = A ordered or unordered list of data points (int or float).
        left = Q-test of minimum value in the ordered list if True.
        right = Q-test of maximum value in the ordered list if True.
        q_dict = A dictionary of Q-values for a given confidence level,
            where the dict. keys are sample sizes N, and the associated values
            are the corresponding critical Q values. E.g.,
            {3: 0.97, 4: 0.829, 5: 0.71, 6: 0.625, ...}

    Returns a list of 2 values for the outliers, or None.
    E.g.,
       for [1,1,1] -> [None, None]
       for [5,1,1] -> [None, 5]
       for [5,1,5] -> [1, None]

    """
    assert(left or right), 'At least one of the variables, `left` or `right`,␣
↪must be True.'
    assert(len(data) >= 3), 'At least 3 data points are required'
    assert(len(data) <= max(q_dict.keys())), 'Sample size too large'

    sdata = sorted(data)
    Q_mindiff, Q_maxdiff = (0,0), (0,0)

    if left:
        Q_min = (sdata[1] - sdata[0])
        try:
            Q_min /= (sdata[-1] - sdata[0])
        except ZeroDivisionError:
            pass
        Q_mindiff = (Q_min - q_dict[len(data)], sdata[0])

    if right:
        Q_max = abs((sdata[-2] - sdata[-1]))
        try:
            Q_max /= abs((sdata[0] - sdata[-1]))
        except ZeroDivisionError:
            pass
        Q_maxdiff = (Q_max - q_dict[len(data)], sdata[-1])
```

```
    if not Q_mindiff[0] > 0 and not Q_maxdiff[0] > 0:
        outliers = [None, None]

    elif Q_mindiff[0] == Q_maxdiff[0]:
        outliers = [Q_mindiff[1], Q_maxdiff[1]]

    elif Q_mindiff[0] > Q_maxdiff[0]:
        outliers = [Q_mindiff[1], None]

    else:
        outliers = [None, Q_maxdiff[1]]

    return outliers
```

1.2 Read in AA Quant data and metadata

[3]:
```
aminos = pd.read_csv(r'data/AA-Results.csv',index_col=[0],header=[0,1])
aminos = aminos.sort_index()

aminos_key = pd.read_csv(r'data/AA-MetaData.csv',index_col=[0],header=[0])

blank=aminos.loc['blank'].melt()
blank.columns=['variable','measure','value']
blank=blank[blank.measure=='Final Conc.']
blank=blank.drop('measure',axis=1)
blank['cond']='blank'
blank
```

[3]:

	variable	value	cond
12	Tryptophan (Trp)-pos Results	0.4153	blank
13	Tryptophan (Trp)-pos Results	0.4160	blank
14	Tryptophan (Trp)-pos Results	0.4309	blank
15	Tryptophan (Trp)-pos Results	0.4151	blank
16	Tryptophan (Trp)-pos Results	0.4114	blank
…	…	…	…
1171	Arginine (Arg)-pos Results	0.2365	blank
1172	Arginine (Arg)-pos Results	0.5347	blank
1173	Arginine (Arg)-pos Results	0.2619	blank
1174	Arginine (Arg)-pos Results	0.2252	blank
1175	Arginine (Arg)-pos Results	0.2366	blank

[300 rows x 3 columns]

2 Plots results, using Q-test to filter out outliers

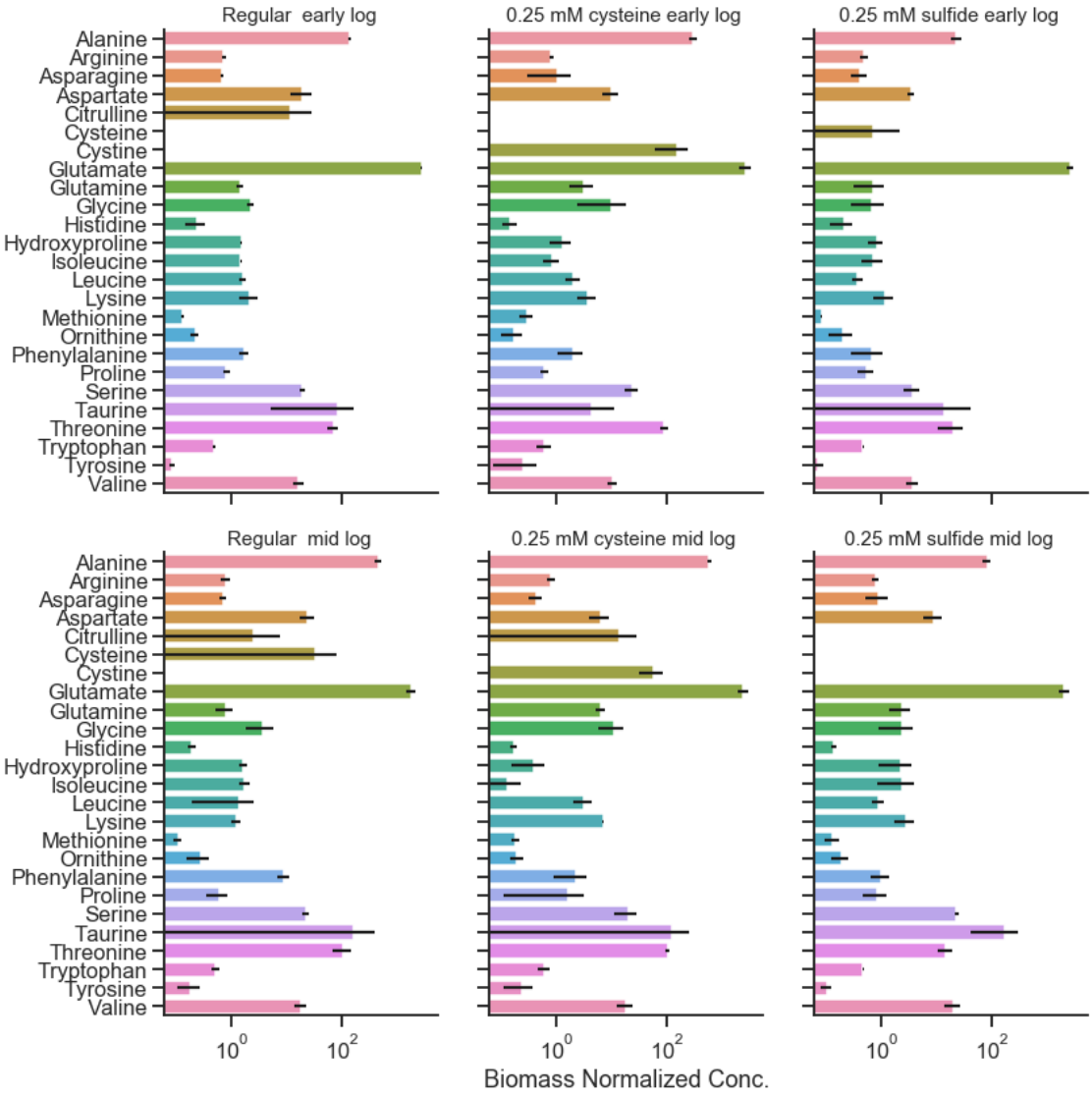
- Creates table df_conc, which is used to generate the subsequent figure

- Must run this code for the following segment to work (uses df_conc)

[4]:
```
cols = [(x,'Final Conc.') for x in aminos.columns.levels[0].values]
fig,ax = plt.subplots(2,3,figsize=(12,12),sharex=True,sharey=True)
i=0
j=0
df_conc=pd.DataFrame()
for index,df in aminos_key.groupby(by=['Time point','Growth condition']):
    #protein =df['Protein concentration (ug/mL)']
    conc = aminos.loc[df.index.values,cols]
    conc.columns = conc.columns.droplevel(1)

    for col in conc.columns:
        x = conc[col]
        if len(x) >= 3:
            res = np.array(dixon_test(x.values))
            res =res[res!=None]
            if len(res)>0:
                x[x==res[0]]=None
        conc.loc[:,col] = x
    cond=' '.join([index[1].split('(')[0],index[0]])[:-5]
    if '+' in cond:
        cond=cond.split('+')[1][1:]
    means = conc.mean()
    errors = conc.std()
    temp = conc.melt()
    temp['cond']=cond
    df_conc=pd.concat([df_conc,temp])
    labels = [x.split()[0] for x in conc.columns]
    sns.barplot(y=labels,x=means,xerr=errors,ax=ax[j][i%3])
    ax[j][i%3].set_title(cond,fontsize=15)
    i+=1
    if i%3 == 0 and i>0:
        j=1
    if j == 1 and i%3 == 1:
        ax[j][i%3].set_xlabel('Biomass Normalized Conc.')
#ax[j][i%3].set_xlim(-1,50)
ax[j][i%3].set_xscale('log')
fig.tight_layout()
sns.despine()
df_conc=pd.concat([df_conc,blank])
#plt.savefig('AminoAcid-Quant.png',bbox_inches='tight',dpi=100)
```

```
//anaconda3/lib/python3.7/site-packages/ipykernel_launcher.py:52:
RuntimeWarning: invalid value encountered in double_scalars
//anaconda3/lib/python3.7/site-packages/ipykernel_launcher.py:60:
RuntimeWarning: invalid value encountered in double_scalars
//anaconda3/lib/python3.7/site-packages/ipykernel_launcher.py:52:
```

```
RuntimeWarning: invalid value encountered in double_scalars
//anaconda3/lib/python3.7/site-packages/ipykernel_launcher.py:60:
RuntimeWarning: invalid value encountered in double_scalars
//anaconda3/lib/python3.7/site-packages/ipykernel_launcher.py:52:
RuntimeWarning: invalid value encountered in double_scalars
//anaconda3/lib/python3.7/site-packages/ipykernel_launcher.py:60:
RuntimeWarning: invalid value encountered in double_scalars
//anaconda3/lib/python3.7/site-packages/ipykernel_launcher.py:52:
RuntimeWarning: invalid value encountered in double_scalars
//anaconda3/lib/python3.7/site-packages/ipykernel_launcher.py:60:
RuntimeWarning: invalid value encountered in double_scalars
```



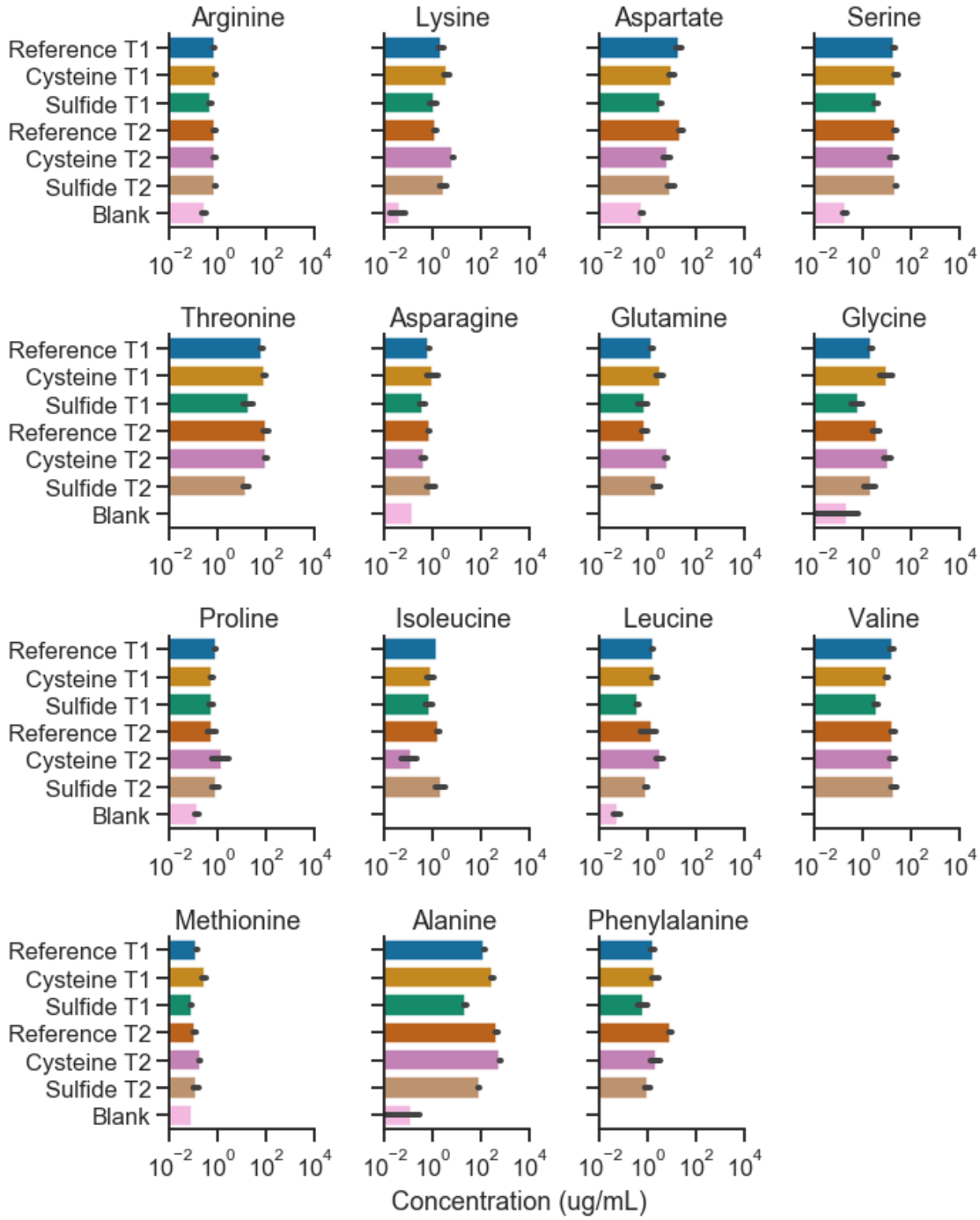
3 Figure 3 C

- Be sure you run the code segments above, as this relies on the generation of df_conc
- Plots results with outliers removed on a log scale

[5]:
```
df_conc=df_conc[(df_conc.variable!='Citrulline - pos Results')&(df_conc.
 ↪variable!='Taurine - pos Results')&(df_conc.variable!='Hydroxyproline␣
 ↪(Hyp)-pos Results')]
cond_map={'Regular  early log ':'Reference T1', '0.25 mM cysteine early log ':
 ↪'Cysteine T1',
       '0.25 mM sulfide early log ': 'Sulfide T1', 'Regular  mid log ':
 ↪'Reference T2',
       '0.25 mM cysteine mid log ':'Cysteine T2', '0.25 mM sulfide mid log ':
 ↪'Sulfide T2','blank':'Blank'}
df_conc['labels']=df_conc.cond.map(cond_map)
rows=4
cols=4
fig=plt.figure(figsize=(cols*2.5,rows*3))
ax=plt.subplot(rows,cols,1)
i=1
order=['Arginine (Arg)-pos Results','Lysine (Lys)-pos Results','Aspartate␣
 ↪(Asp)-pos Results','Serine (Ser)-pos Results',
       'Threonine (Thr)-pos Results', 'Asparagine (Asn)-pos Results','Glutamine␣
 ↪(Gln)-pos Results',
       'Glycine (Gly)-pos Results', 'Proline (Pro)-pos Results',
       'Isoleucine (Ile)-pos Results', 'Leucine (Leu)-pos Results', 'Valine␣
 ↪(Val)-pos Results',
        'Methionine (Met)-pos Results',
        'Alanine (Ala)-pos Results',
        'Phenylalanine (Phe)-pos Results']
for measure in order:
    df = df_conc[df_conc.variable==measure]
    g=sns.barplot(y='labels',x='value',data=df,ax=ax)
    #g.set_xticklabels(g.get_xticklabels(),rotation=90)
    g.set_xscale('log')
    g.set_ylabel('')
    g.set_xlabel('')
    g.set_title(measure.split('(')[0])
    if df['value'].max() > 500:
        g.set_xlim(10,10**4)
        #g.set_xticks(range(1,10**4))
    g.set_xlim(.01,10**4)
    g.set_xticks([10**-2,10**0,10**2,10**4])
    if (i-1)%cols != 0:
        g.set_yticklabels('')
    i+=1
    if i <= len(order):
```

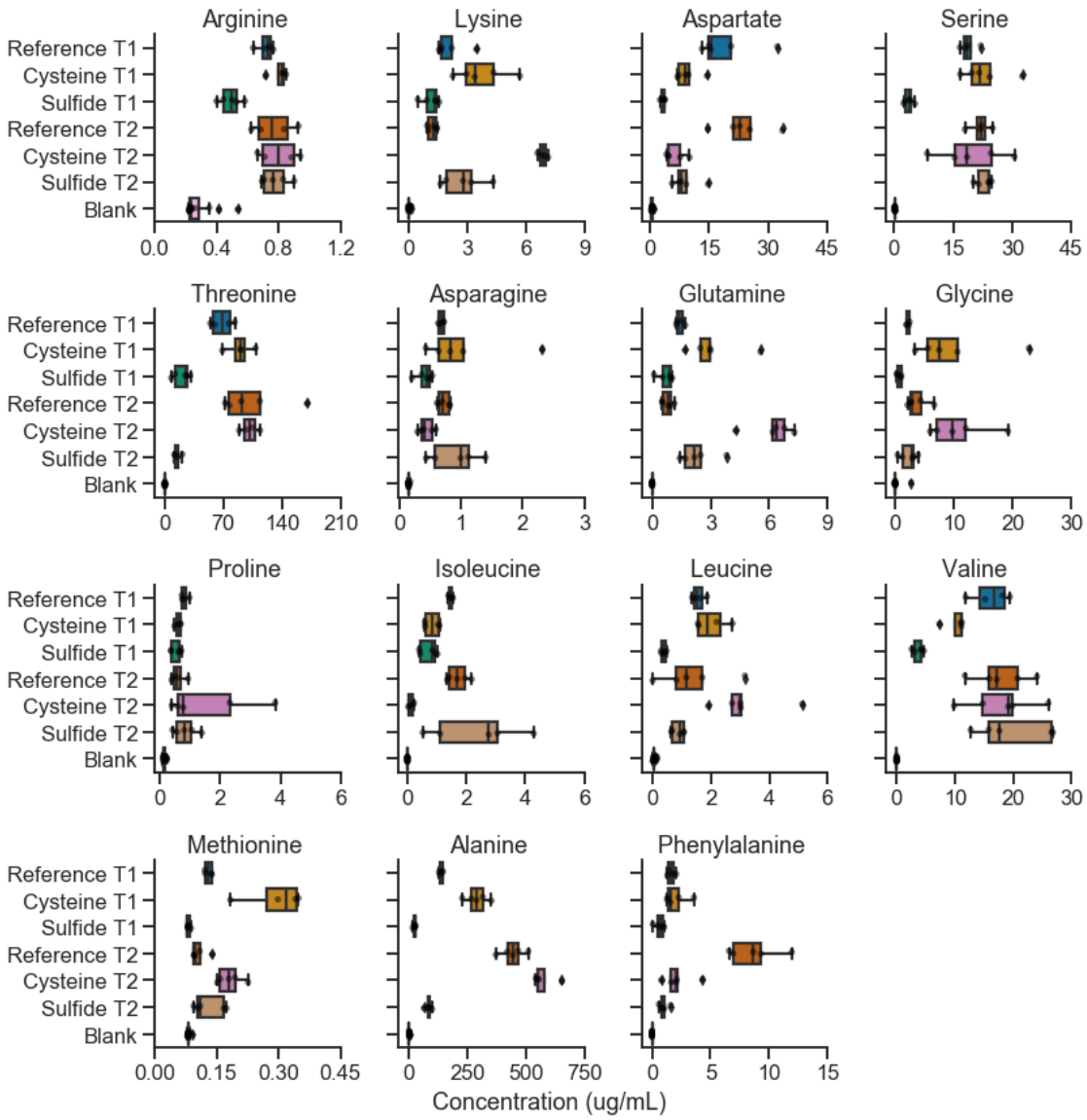
```
        ax=plt.subplot(rows,cols,i)

fig.tight_layout()
fig.text(.4,0,'Concentration (ug/mL)')
#plt.savefig('2020_Targeted_aminos3.pdf',bbox_inches='tight')
```

[5]: Text(0.4, 0, 'Concentration (ug/mL)')



[105]:
```
df_conc=df_conc[(df_conc.variable!='Citrulline - pos Results')&(df_conc.
 ↪variable!='Taurine - pos Results')&(df_conc.variable!='Hydroxyproline␣
 ↪(Hyp)-pos Results')]
cond_map={'Regular  early log ':'Reference T1', '0.25 mM cysteine early log ':
 ↪'Cysteine T1',
       '0.25 mM sulfide early log ': 'Sulfide T1', 'Regular  mid log ':
 ↪'Reference T2',
       '0.25 mM cysteine mid log ':'Cysteine T2', '0.25 mM sulfide mid log ':
 ↪'Sulfide T2','blank':'Blank'}
df_conc['labels']=df_conc.cond.map(cond_map)
rows=4
cols=4
fig=plt.figure(figsize=(cols*3,rows*3))
ax=plt.subplot(rows,cols,1)
i=1
order=['Arginine (Arg)-pos Results','Lysine (Lys)-pos Results','Aspartate␣
 ↪(Asp)-pos Results','Serine (Ser)-pos Results',
       'Threonine (Thr)-pos Results', 'Asparagine (Asn)-pos Results','Glutamine␣
 ↪(Gln)-pos Results',
       'Glycine (Gly)-pos Results', 'Proline (Pro)-pos Results',
       'Isoleucine (Ile)-pos Results', 'Leucine (Leu)-pos Results', 'Valine␣
 ↪(Val)-pos Results',
        'Methionine (Met)-pos Results',
        'Alanine (Ala)-pos Results',
        'Phenylalanine (Phe)-pos Results']
for measure in order:
    df = df_conc[df_conc.variable==measure]
    g=sns.boxplot(y='labels',x='value',data=df,ax=ax)
    sns.stripplot(y='labels',x='value',data=df,ax=ax,color='black',alpha=0.5)
    #g.set_xticklabels(g.get_xticklabels(),rotation=90)
    #g.set_xscale('log')
    g.set_ylabel('')
    g.set_xlabel('')
    g.set_title(measure.split('(')[0])

    #if df['value'].max() > 500:
        #g.set_xlim(10,10**4)
        #g.set_xticks(range(1,10**4))
    #g.set_xlim(.01,10**2)
    #g.set_xticks([10**-3,10**-2,10**0,10**2])
    if df.value.dropna().max() < 10 and df.value.dropna().max()>1:
        if df.value.dropna().max() < 6:
            if df.value.dropna().max() < 3:
                g.set_xticks([0,1,2,3])
```

```
            else:
                g.set_xticks([0,2,4,6])
        else:
            g.set_xticks([0,3,6,9])
    if df.value.dropna().max() > 10 and df.value.dropna().max()<100:
        if df.value.dropna().max() < 30:
            if df.value.dropna().max() < 15:
                g.set_xticks([0,5,10,15])
            else:
                g.set_xticks([0,10,20,30])
        else:
            g.set_xticks([0,15,30,45])
    if df.value.dropna().max() >100:
        if df.value.dropna().max() >500:
            g.set_xticks([0,250,500,750])
        else:
            g.set_xticks([0,70,140,210])
    if df.value.dropna().max() <2:
        if df.value.dropna().max() <.5:
            g.set_xticks([0,.15,.3,.45])
        else:
            g.set_xticks([0,.4,.8,1.2])
    if (i-1)%cols != 0:
        g.set_yticklabels('')
    i+=1
    if i <= len(order):
        ax=plt.subplot(rows,cols,i)
fig.tight_layout()
fig.text(.4,0,'Concentration (ug/mL)')
plt.savefig('2020_Targeted_aminos_boxplot.pdf',bbox_inches='tight')
```



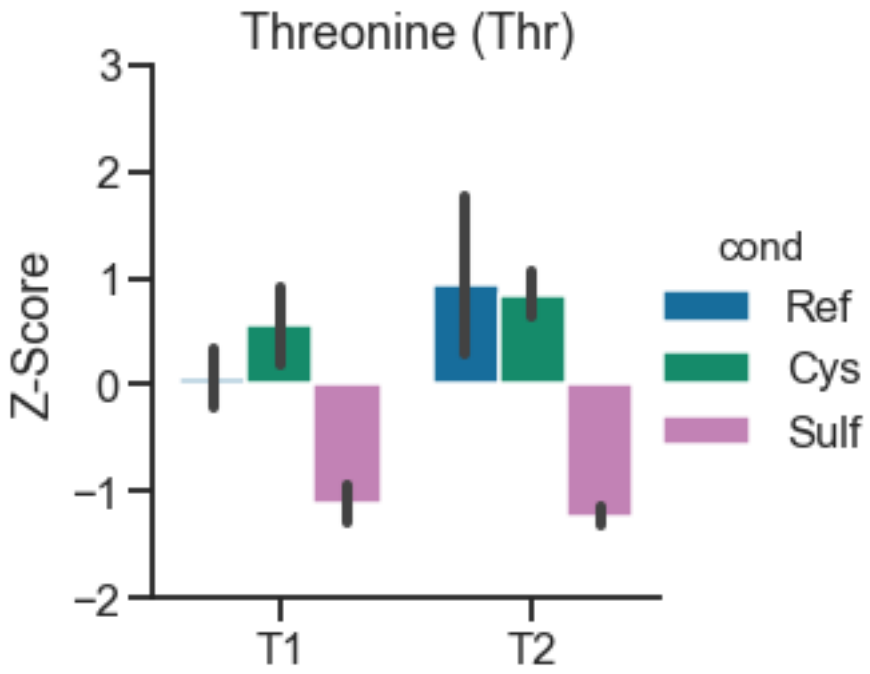
4 Figure 4 C

- Requires above code to be run first (Fig 3 C)

[6]:
```
c=sns.color_palette('colorblind')
c=[c[0],c[2],c[4]]
order=['Threonine (Thr)-pos Results',
       'Isoleucine (Ile)-pos Results', 'Leucine (Leu)-pos Results', 'Valine␣
↪(Val)-pos Results']
for measure in order:
    df = df_conc[df_conc.variable==measure].dropna()
```

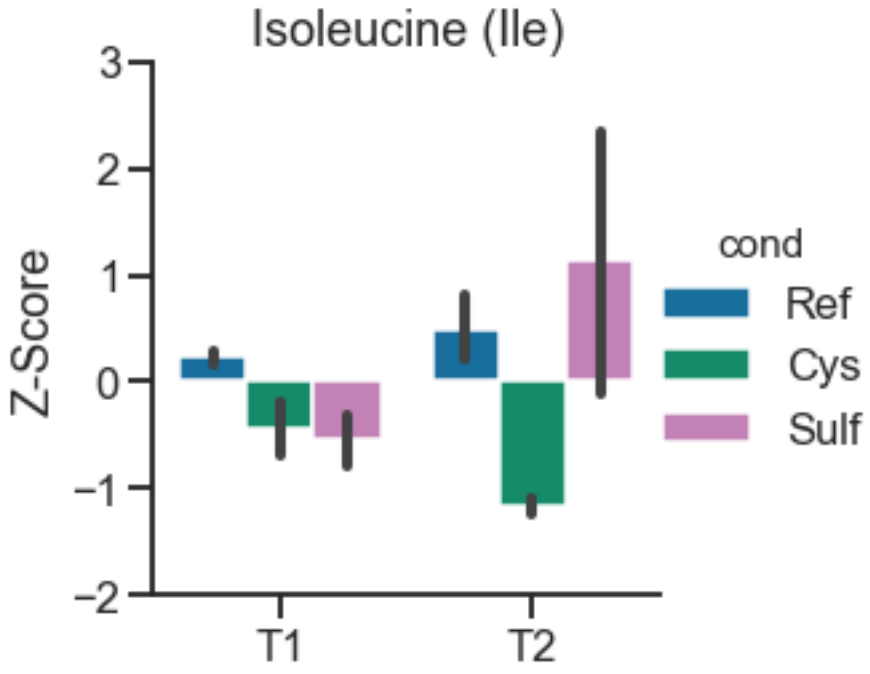
```
    df['cond']=df['cond'].map(cond_map)
    df=df[df['cond']!='Blank']
    t=[]
    cond=[]
    for val in df['cond']:
        val=val.split()
        if val[0] !='Sulfide':
            cond.append(val[0][:3])
        else:
            cond.append(val[0][:4])
        t.append(val[1])
    df['cond']=cond
    df['t']=t

    df['Z-Score']=zscore(df['value'].values)
    plt.figure(figsize=(4,4))
    sns.
↪catplot(x='t',hue='cond',y='Z-Score',data=df,palette=c,kind='bar',height=4)
    #plt.legend(loc=2,bbox_to_anchor=(1,1))
    plt.xlabel('')
    plt.yticks([-2,-1,0,1,2,3])
    plt.title(measure.split('-')[0])
    #plt.savefig(measure.split('-')[0]+'-zscore.pdf',bbox_inches='tight')
    plt.show()
    plt.clf()
```

```
<Figure size 288x288 with 0 Axes>
```



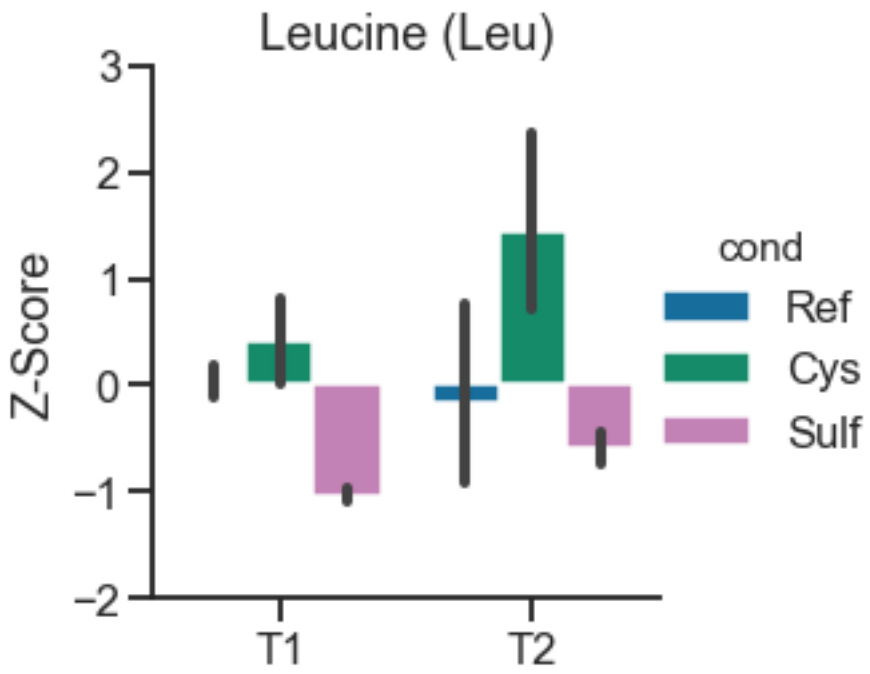
<Figure size 432x288 with 0 Axes>

<Figure size 288x288 with 0 Axes>



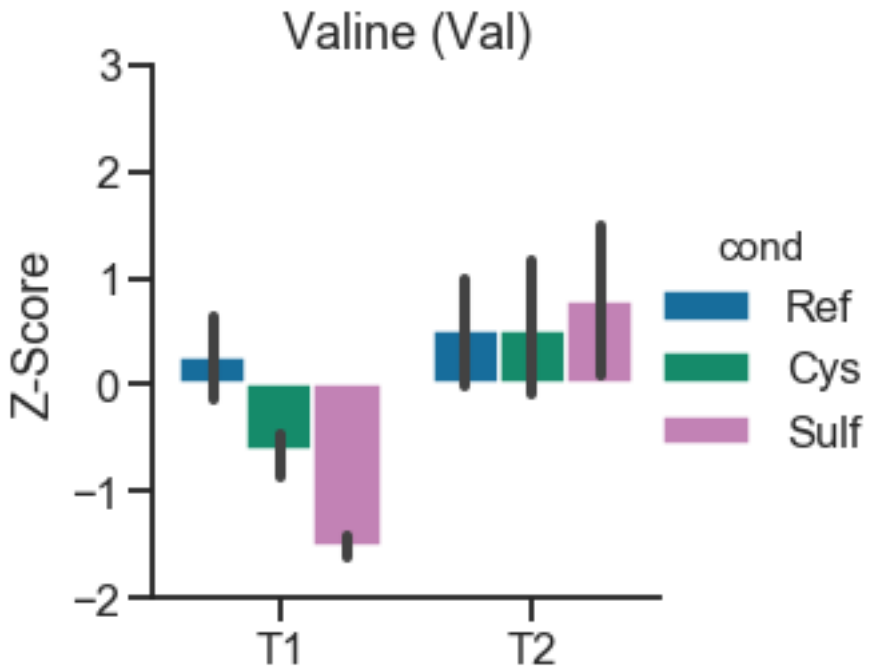
```
<Figure size 432x288 with 0 Axes>
```

```
<Figure size 288x288 with 0 Axes>
```




```
<Figure size 432x288 with 0 Axes>
```

```
<Figure size 288x288 with 0 Axes>
```




```
<Figure size 432x288 with 0 Axes>
```

5 Untargeted Metabolomics

- Results tables used below were generated by XCMS online

6 Figure 4 B

- XCMS online systems biology results
- Provides pathway dysregulation for multi sample comparison (cys vs reg vs sulf)

[144]:
```
sysbio = pd.read_csv(r'data/Untargeted_Met_SysBioRes_All.tsv',sep='\t')
sysbio = sysbio[sysbio['p-values']<=.01]
sysbio['q-values']=multipletests(sysbio['p-values'], method='fdr_bh',alpha=0.
↪01)[:2][1]
sysbio['logp'] = -np.log(sysbio['p-values'])
sysbio = sysbio[sysbio['All metabolites2*']>=4]
sysbio['overlap'] = 100*sysbio['Overlapping putative metabolites1']/sysbio['All␣
↪metabolites2*']
sysbio = sysbio.sort_values(by=['logp','overlap'],ascending=False)
sysbio
```

[144]:

	Pathway	Overlapping genes	Overlapping genes.1	All genes	Overlapping proteins	Overlapping proteins.1	All proteins	Overlapping putative metabolites1
0	isoleucine biosynthesis I (from threonine)	0	0%	0	0	0%	0	6
2	valine biosynthesis	0	0%	0	0	0%	0	3
3	incomplete reductive TCA cycle	0	0%	0	0	0%	0	4
4	2-oxopentenoate degradation	0	0%	0	0	0%	0	3
5	phosphopantothenate biosynthesis I	0	0%	0	0	0%	0	
6	respiration (anaerobic)	0	0%	0	0	0%	0	
9	glyoxylate cycle	0	0%	0	0	0%	0	
10	mixed acid fermentation	0	0%	0	0	0%	0	
11	catechol degradation to 2-oxopent-4-enoate I	0	0%	0	0	0%	0	
12	threonine degradation III (to methylglyoxal)	0	0%	0	0	0%	0	
13	pyrimidine ribonucleosides salvage I	0	0%	0	0	0%	0	
14	UDP-N-acetyl- -D-mannosaminouronate biosynthesis	0	0%	0	0	0%	0	
15	methylerythritol phosphate pathway II	0	0%	0	0	0%	0	
16	\'\"pyridoxal 5\'\'-phosphate salvage pathway\"\'	0	0%	0	0	0%	0	
17	gentisate degradation	0	0%	0	0	0%	0	
18	biotin biosynthesis from 7-keto-8-aminopelargo…	0	0%	0	0	0%	0	
21	TCA cycle IV (2-oxoglutarate decarboxylase)	0	0%	0	0	0%	0	
19	TCA cycle III (helicobacter)	0	0%	0	0	0%	0	
20	lysine biosynthesis I	0	0%	0	0	0%	0	

5	0%	0	3
6	0%	0	4
9	0%	0	3
10	0%	0	4
11	0%	0	2
12	0%	0	2
13	0%	0	2
14	0%	0	2
15	0%	0	3
16	0%	0	2
17	0%	0	2
18	0%	0	2
21	0%	0	4
19	0%	0	3
20	0%	0	3

	Overlapping putative metabolites1.1	All metabolites2*	p-values	q-values	\
0	54.5%	11	0.00068	0.00660	
2	60.0%	5	0.00120	0.00660	
3	40.0%	10	0.00120	0.00660	
4	42.9%	7	0.00200	0.00792	
5	37.5%	8	0.00260	0.00792	
6	26.7%	15	0.00300	0.00792	
9	30.0%	10	0.00430	0.00792	
10	23.5%	17	0.00440	0.00792	
11	50.0%	4	0.00540	0.00792	
12	50.0%	4	0.00540	0.00792	
13	50.0%	4	0.00540	0.00792	
14	50.0%	4	0.00540	0.00792	
15	25.0%	12	0.00700	0.00890	
16	40.0%	5	0.00790	0.00890	
17	40.0%	5	0.00790	0.00890	
18	40.0%	5	0.00790	0.00890	
21	30.8%	13	0.00890	0.00890	
19	23.1%	13	0.00890	0.00890	
20	23.1%	13	0.00890	0.00890	

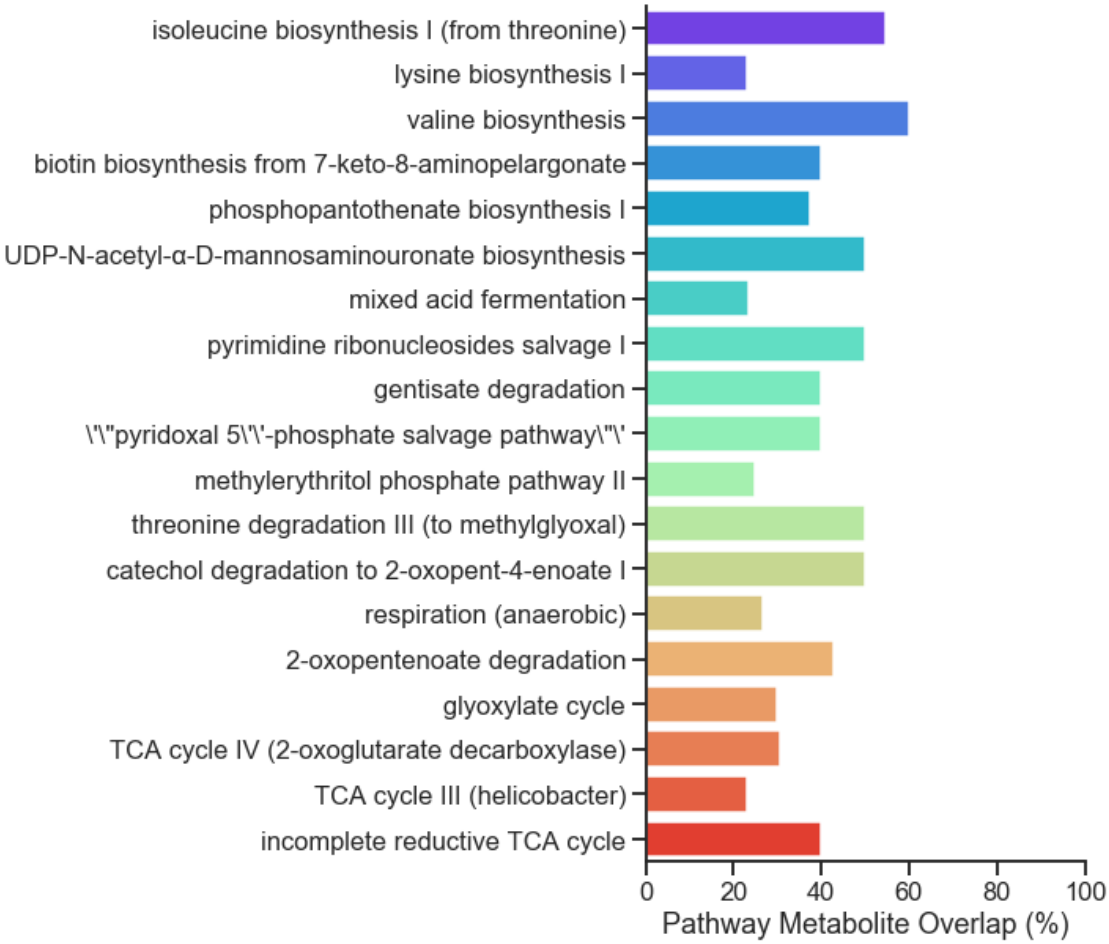
	logp	overlap
0	7.293418	54.545455
2	6.725434	60.000000
3	6.725434	40.000000
4	6.214608	42.857143
5	5.952244	37.500000
6	5.809143	26.666667
9	5.449140	30.000000
10	5.426151	23.529412
11	5.221356	50.000000

```
12  5.221356  50.000000
13  5.221356  50.000000
14  5.221356  50.000000
15  4.961845  25.000000
16  4.840893  40.000000
17  4.840893  40.000000
18  4.840893  40.000000
21  4.721704  30.769231
19  4.721704  23.076923
20  4.721704  23.076923
```

[ ]:

7 Names and ordering were further curated using Adobe illustrator

[147]:
```
plt.figure(figsize=(5,10))
temp = sysbio[(sysbio['p-values']<=.01)&(sysbio['overlap']>=20)]
sort=[]
for p in temp['Pathway']:
    if 'lysine' in p.lower() or 'valine' in p.lower() or 'isoleucine' in p.
 ↪lower():
        sort.append('bcaa')
    elif 'biosynthesis' in p.lower():
        sort.append('biosynth')
    elif 'degredation' in p.lower():
        sort.append('deg')
    elif 'glyoxylate' in p.lower() or 'tca' in p.lower():
        sort.append('tca')
    elif 'fermentation' in p.lower():
        sort.append('ferm')
    else:
        sort.append('path')
temp['sort']=sort
sns.barplot(y='Pathway',x='overlap',data=temp.
 ↪sort_values(by='sort'),palette='rainbow')
plt.ylabel('')
plt.xlabel('Pathway Metabolite Overlap (%)')

plt.xlim(0,100)
plt.savefig('Metabolite-Pathway-Disregulation.pdf',bbox_inches='tight')
```



8 XCMS Online MultiClass Results (mummichog + feature abundances)

[149]:
```
pathway = pd.read_csv(r'data/pathwayanalysis_mummichog_all.csv')
features=pd.read_csv(r'data/XCMS-MultiClass-All.csv',index_col=0)
features
```

[149]:

featureidx	name	mzmed	mzmin	mzmax	rtmed	\
1	M911T2_5	911.486746	911.486356	911.489983	2.049583	
2	M485T23_1	484.638683	484.638013	484.639495	22.604533	
3	M514T23_1	514.147566	514.147004	514.148278	22.626633	
4	M515T23_1	514.649161	514.648714	514.649834	22.625600	
5	M969T23	969.280946	969.279960	969.282163	22.613367	
…	…	…	…	…	…	
29983	M979T20_4	979.031523	979.026895	979.034959	19.580333	

```
29984       M1061T20_4  1061.034120  1061.030956  1061.038579  19.574900
29985        M567T19_3   566.980125   566.974108   566.980818  19.285067
29986       M1059T20_3  1059.047693  1059.044559  1059.053117  19.578500
29987        M939T20_1   938.532292   938.528914   938.534355  19.569067

                rtmin      rtmax  npeaks  Cys_early  Cys_mid  …  \
featureidx                                                     …
1            2.049097   2.059120       3          0        0  …
2           22.441633  22.768150      21          4        0  …
3           22.450200  22.784867      23          5        0  …
4           22.450200  22.751433      19          4        0  …
5           22.441633  22.793250      24          5        0  …
…                 …          …       …          …        …  …
29983       19.436200  19.880550      30          5        5  …
29984       19.429500  19.682433      29          4        5  …
29985       18.955300  19.505383      35          5        4  …
29986       19.454600  19.634017      29          4        5  …
29987       19.437800  19.657700      29          4        5  …

                 Sulf_4         Sulf_5         Sulf_6         Sulf_7  \
featureidx
1                0.000000       0.000000       0.000000       0.000000
2             2947.131112    5387.786899   10512.508700    9942.933647
3            10228.620210   12739.203810   24752.718460   25444.025700
4             1473.240383    3588.953005    8237.769879    8845.544354
5             5398.305192    7712.465577   13713.700000   14791.932000
…                    …              …              …              …
29983        74186.842800   34938.100800   36523.133400   33786.608000
29984        66480.864000   30224.658160   32453.198770   23956.123900
29985        37974.118000  190839.678200  180341.841400  153501.735500
29986       119789.280000   28941.711040   36981.664000   21502.682530
29987        38812.020000   15867.681950   20405.040330   15053.517070

                 Sulf_8       Sulf_12       Sulf_13        Sulf_14  \
featureidx
1                0.000000       0.00000       0.00000       0.000000
2            11833.788420   37872.27600   41237.29200   33370.244970
3            25487.200520   65906.34182   74647.62785   67433.193000
4             8302.467224   28255.61148   29283.38952   26005.117800
5            14893.339120   47359.63124   54289.95368   48602.328000
…                    …              …             …              …
29983        56593.479310   50883.76055   31963.93469   22824.688860
29984        54586.832500   54786.08250   17157.27391   21367.899470
29985       153928.292800  165492.47160  187036.36970  157449.180500
29986        69536.730160   71331.79050   17133.32492   16081.842730
29987        33345.775640   30625.96364   10903.55063    8757.898971
```

```
                 Sulf_15       Sulf_16
featureidx
1                0.00000       0.00000
2            44785.12589   57506.10800
3            83768.10200   95294.30221
4            33901.81809   40221.23230
5            56710.08310   68136.46000
…                    …             …
29983        52402.77692  100848.12300
29984        42938.66087   97891.91788
29985       171034.72650   86020.71200
29986        54714.21035  169284.58920
29987        28008.05973   56703.22411

[29987 rows x 61 columns]
```

9 Extract feature ID and compound mapping

[150]:
```
cmp_dict={}
temp = pathway.loc[:,['overlap_features(id)','overlap_input(featureidx)']].
 ↪dropna()
for index in temp.index:
    names = temp.loc[index,'overlap_features(id)']
    ids =temp.loc[index,'overlap_input(featureidx)']
    for name, i in zip(names.split(';'),ids.split(';')):
        if ',' in i:
                cmp_dict[name]=[int(x) for x in i.split(',')]
        else:
            cmp_dict[name]=int(i)
cmp_dict
```

[150]:
```
{'CPD-15056': 68,
 '2-ACETO-2-HYDROXY-BUTYRATE': 234,
 'PYRUVATE': [117, 241, 174, 197, 42, 298, 102, 34],
 '1-KETO-2-METHYLVALERATE': [213, 44],
 '2-KETO-3-METHYL-VALERATE': [179, 38],
 'CPD-16013': 68,
 'GLYCEROL-3P': [100, 303],
 'GLYCEROL': 44,
 'CMP': 53,
 '2-KETO-ISOVALERATE': 38,
 '2-ACETO-LACTATE': [161, 184, 234, 158],
 'FUM': 220,
 'SUC': 100,
 'MAL': [220, 241],
 '4-HYDROXY-2-KETOVALERATE': [161, 184, 234, 158],
```

```
'OXOPENTENOATE': [158, 161],
'2-DEHYDROPANTOATE': 234,
'L-PANTOATE': [213, 44],
'GLYOX': 241,
'CPD-14965': 239,
'AMINO-OXOBUT': 27,
'METHYL-GLYOXAL': 184,
'UMP': 289,
'UDP-N-ACETYL-D-GLUCOSAMINE': [115, 296],
'UDP-MANNAC': [115, 296],
'HYDROXY-METHYL-BUTENYL-DIP': 110,
'PYRIDOXAL': 239,
'PYRIDOXINE': 113,
'DETHIOBIOTIN': 111,
'S-ADENOSYLMETHIONINE': 242,
'DELTA1-PIPERIDEINE-2-6-DICARBOXYLATE': [39, 21],
'AMINO-HYDROXYMETHYL-METHYL-PYR-P': 86,
'CHORISMATE': 188,
'ASCORBATE': [34, 117, 197, 298, 42, 174, 102],
'CPD-329': [34, 117, 197, 298, 42, 174, 102],
'2-AMINOACRYLATE': [68, 262],
'OROTATE': 247,
'GLUCOSAMINE-1P': 171,
'2-D-THREO-HYDROXY-3-CARBOXY-ISOCAPROATE': 38,
'2K-4CH3-PENTANOATE': [179, 38],
'3-CARBOXY-3-HYDROXY-ISOCAPROATE': 38,
'ACETYL-GLU': [49, 21],
'3OH-4P-OH-ALPHA-KETOBUTYRATE': [153, 96],
'TMP': 210,
'CPD-10754': [101, 233],
'CPD-13722': 234,
'CPD-180': 171,
'PREPHENATE': 188,
'HCN': 262,
'BUTYRIC_ACID': [67, 71, 213],
'XANTHOSINE': 254,
'D-XYLULOSE': [161, 158, 184],
'DCMP': 210,
'CPD-7682': 215,
'CPD0-1159': 312,
'DTDP-D-GLUCOSE': 280,
'SULFATE': 79,
'PSEUDOURIDINE-5-P': 289,
'ACRYLATE': 184,
'DODECANOATE': 105}
```

[151]:
```
rev_cmp_dict ={}
for key in cmp_dict:
    idx = cmp_dict[key]
    if type(idx) != type([]):
        idx = [idx]
    for i in idx:
        if i not in rev_cmp_dict:
            rev_cmp_dict[i]=[key]
        else:
            rev_cmp_dict[i].append(key)
rev_cmp_dict
```

[151]:
```
{68: ['CPD-15056', 'CPD-16013', '2-AMINOACRYLATE'],
 234: ['2-ACETO-2-HYDROXY-BUTYRATE',
  '2-ACETO-LACTATE',
  '4-HYDROXY-2-KETOVALERATE',
  '2-DEHYDROPANTOATE',
  'CPD-13722'],
 117: ['PYRUVATE', 'ASCORBATE', 'CPD-329'],
 241: ['PYRUVATE', 'MAL', 'GLYOX'],
 174: ['PYRUVATE', 'ASCORBATE', 'CPD-329'],
 197: ['PYRUVATE', 'ASCORBATE', 'CPD-329'],
 42: ['PYRUVATE', 'ASCORBATE', 'CPD-329'],
 298: ['PYRUVATE', 'ASCORBATE', 'CPD-329'],
 102: ['PYRUVATE', 'ASCORBATE', 'CPD-329'],
 34: ['PYRUVATE', 'ASCORBATE', 'CPD-329'],
 213: ['1-KETO-2-METHYLVALERATE', 'L-PANTOATE', 'BUTYRIC_ACID'],
 44: ['1-KETO-2-METHYLVALERATE', 'GLYCEROL', 'L-PANTOATE'],
 179: ['2-KETO-3-METHYL-VALERATE', '2K-4CH3-PENTANOATE'],
 38: ['2-KETO-3-METHYL-VALERATE',
  '2-KETO-ISOVALERATE',
  '2-D-THREO-HYDROXY-3-CARBOXY-ISOCAPROATE',
  '2K-4CH3-PENTANOATE',
  '3-CARBOXY-3-HYDROXY-ISOCAPROATE'],
 100: ['GLYCEROL-3P', 'SUC'],
 303: ['GLYCEROL-3P'],
 53: ['CMP'],
 161: ['2-ACETO-LACTATE',
  '4-HYDROXY-2-KETOVALERATE',
  'OXOPENTENOATE',
  'D-XYLULOSE'],
 184: ['2-ACETO-LACTATE',
  '4-HYDROXY-2-KETOVALERATE',
  'METHYL-GLYOXAL',
  'D-XYLULOSE',
  'ACRYLATE'],
 158: ['2-ACETO-LACTATE',
```

```
 '4-HYDROXY-2-KETOVALERATE',
 'OXOPENTENOATE',
 'D-XYLULOSE'],
220: ['FUM', 'MAL'],
239: ['CPD-14965', 'PYRIDOXAL'],
27: ['AMINO-OXOBUT'],
289: ['UMP', 'PSEUDOURIDINE-5-P'],
115: ['UDP-N-ACETYL-D-GLUCOSAMINE', 'UDP-MANNAC'],
296: ['UDP-N-ACETYL-D-GLUCOSAMINE', 'UDP-MANNAC'],
110: ['HYDROXY-METHYL-BUTENYL-DIP'],
113: ['PYRIDOXINE'],
111: ['DETHIOBIOTIN'],
242: ['S-ADENOSYLMETHIONINE'],
39: ['DELTA1-PIPERIDEINE-2-6-DICARBOXYLATE'],
21: ['DELTA1-PIPERIDEINE-2-6-DICARBOXYLATE', 'ACETYL-GLU'],
86: ['AMINO-HYDROXYMETHYL-METHYL-PYR-P'],
188: ['CHORISMATE', 'PREPHENATE'],
262: ['2-AMINOACRYLATE', 'HCN'],
247: ['OROTATE'],
171: ['GLUCOSAMINE-1P', 'CPD-180'],
49: ['ACETYL-GLU'],
153: ['3OH-4P-OH-ALPHA-KETOBUTYRATE'],
96: ['3OH-4P-OH-ALPHA-KETOBUTYRATE'],
210: ['TMP', 'DCMP'],
101: ['CPD-10754'],
233: ['CPD-10754'],
67: ['BUTYRIC_ACID'],
71: ['BUTYRIC_ACID'],
254: ['XANTHOSINE'],
215: ['CPD-7682'],
312: ['CPD0-1159'],
280: ['DTDP-D-GLUCOSE'],
79: ['SULFATE'],
105: ['DODECANOATE']}
```

10 Unique feature indexes mapping to a metabolite

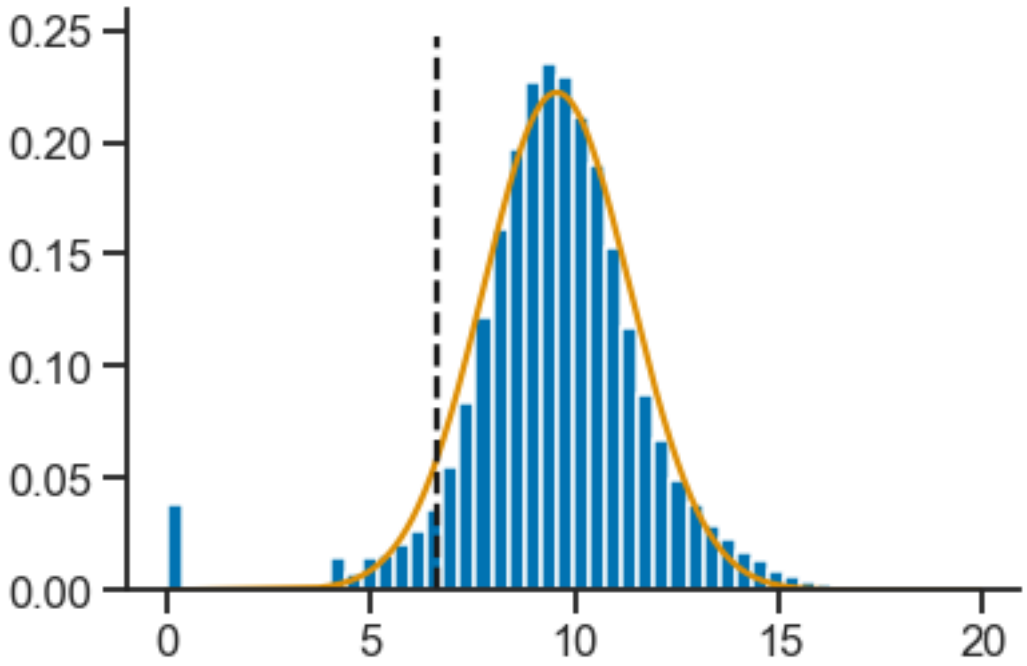
[152]:
```
idxs = []
for key in cmp_dict:
    if type(cmp_dict[key]) == type(1):
        idxs.append(cmp_dict[key])
    else:
        idxs+=cmp_dict[key]
idxs=pd.unique(idxs)
idxs
```

[152]:
```
array([ 68, 234, 117, 241, 174, 197,  42, 298, 102,  34, 213,  44, 179,
         38, 100, 303,  53, 161, 184, 158, 220, 239,  27, 289, 115, 296,
        110, 113, 111, 242,  39,  21,  86, 188, 262, 247, 171,  49, 153,
         96, 210, 101, 233,  67,  71, 254, 215, 312, 280,  79, 105])
```

11 Feature abundance normalization

- Convert Area to Log Area
- Convert Log Area to Z-score

[153]:
```
logarea = pd.DataFrame(index=features.index,columns=features.columns[31:
 ↪],data=features.iloc[:,31:])
logarea=np.log(logarea+1).dropna()

vals = logarea.values.ravel()
hist = plt.hist(vals,bins=50,density=True)
dist = norm(loc=np.median(vals),scale=np.std(vals)/1.25)
plt.plot(sorted(vals),dist.pdf(sorted(vals)))

keep = []
toss = []
cutoff = 0.05
x = dist.ppf(cutoff)
plt.plot((x,x),plt.ylim(),'k--')
for i in logarea.index:
    vals = logarea.loc[i,:].values
    truth = dist.cdf(vals)>cutoff
    if len(truth[truth==True])/len(vals)>.5:
        keep.append(i)
    else:
        toss.append(i)
logarea=logarea.loc[keep,:]
```



[154]:
```
cols = ['Cys_1', 'Cys_1', 'Cys_1', 'Cys_1', 'Cys_1', 'Cys_2', 'Cys_2', 'Cys_2',
       'Cys_2', 'Cys_2', 'Reg_1', 'Reg_1', 'Reg_1', 'Reg_1', 'Reg_1',
       'Reg_2', 'Reg_2', 'Reg_2', 'Reg_2', 'Reg_2', 'Sulf_1', 'Sulf_1',
       'Sulf_1', 'Sulf_1', 'Sulf_1', 'Sulf_2', 'Sulf_2', 'Sulf_2',
       'Sulf_2', 'Sulf_2']
cols = [(x.split('_')[0],x.split('_')[1]) for x in cols]

temp = logarea.copy()
temp.columns = pd.MultiIndex.from_tuples(cols)
avglogarea = temp.groupby(level=[0,1],axis=1).median()
zscores = temp.groupby(level=[0,1],axis=1).median().T.apply(zscore).T
zscores
```

[154]:

	Cys		Reg		Sulf	
	1	2	1	2	1	2
featureidx						
2	0.139815	-2.077243	0.885792	0.776001	-0.209111	0.484747
3	-0.133942	-1.838233	1.128196	0.922728	-0.527024	0.448275
4	0.115952	-2.076387	0.906507	0.780861	-0.184705	0.457773
5	0.026579	-1.984705	1.013676	0.749986	-0.377800	0.572265
6	0.105237	-1.870644	1.060993	0.790479	-0.649394	0.563329
…	…	…	…	…	…	…
29983	1.081408	-0.302608	-0.095585	0.099708	-1.876962	1.094039
29984	1.083481	-0.375817	0.157349	0.148293	-1.942739	0.929433
29985	0.470406	1.072179	-1.822936	-0.776935	0.260781	0.796505

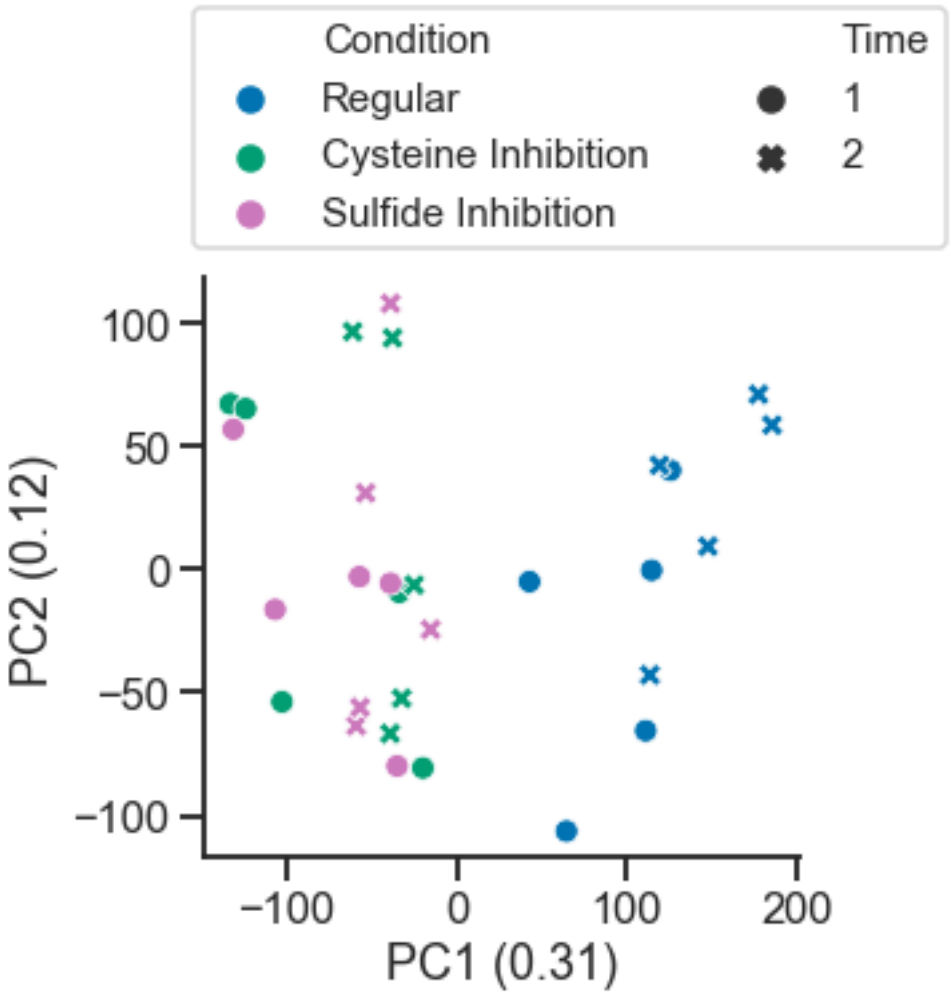
```
29986       1.151228 -0.782653  0.520241 -0.458928 -1.535637  1.105749
29987       1.381079 -0.660045 -0.857043  0.257514 -1.254223  1.132718

[28508 rows x 6 columns]
```

12 Supplementary Figure 4C: PCA Analysis with all features

[16]:
```
colors=sns.color_palette('colorblind')
cols = ['Cys_1', 'Cys_1', 'Cys_1', 'Cys_1', 'Cys_1', 'Cys_2', 'Cys_2', 'Cys_2',
       'Cys_2', 'Cys_2', 'Reg_1', 'Reg_1', 'Reg_1', 'Reg_1', 'Reg_1',
       'Reg_2', 'Reg_2', 'Reg_2', 'Reg_2', 'Reg_2', 'Sulf_1', 'Sulf_1',
       'Sulf_1', 'Sulf_1', 'Sulf_1', 'Sulf_2', 'Sulf_2', 'Sulf_2',
       'Sulf_2', 'Sulf_2']
cols = [(x.split('_')[0],x.split('_')[1]) for x in cols]
temp = logarea.copy()
temp.columns = pd.MultiIndex.from_tuples(cols)
temp=temp[['Reg','Cys','Sulf']]
logarea2 = temp
pca_data =temp.copy().T
pca_data=pca_data.apply(zscore)
pca = PCA(n_components=2)
color=sns.color_palette('colorblind')
c=[colors[0],colors[2],colors[4]]
pca_coord = pca.fit_transform(pca_data)
pca_coord= pd.DataFrame(pca_coord,index=pca_data.index,columns=['PC1','PC2'])
pca_coord = pca_coord.reset_index()
pca_coord.columns=['Condition','Time','PC1','PC2']
labels = {'Cys':'Cysteine Inhibition','Reg':'Regular','Sulf':'Sulfide␣
 ↪Inhibition'}
pca_coord['Condition']=pca_coord['Condition'].map(labels)
print('Explained Variance... PC1:%s, PC2:%s'%tuple(pca.
 ↪explained_variance_ratio_))
loadings = pd.DataFrame(pca.components_,index=['PC1','PC2'],columns=pca_data.
 ↪columns)

plt.figure(figsize=(4,4))
sns.
 ↪scatterplot(x='PC1',y='PC2',hue='Condition',style='Time',data=pca_coord,palette=c)
plt.legend(bbox_to_anchor=(-.05,1.5),ncol=2,loc=2,fontsize=15)
plt.xlabel('PC1 (%s)'%(pca.explained_variance_ratio_[0].round(2)))
plt.ylabel('PC2 (%s)'%(pca.explained_variance_ratio_[1].round(2)))
#plt.savefig('Pred_Metabolites_PCA_All.pdf',bbox_inches='tight')
```

```
Explained Variance… PC1:0.30613375327757675, PC2:0.12063218765258428
```

[16]:
```
Text(0, 0.5, 'PC2 (0.12)')
```



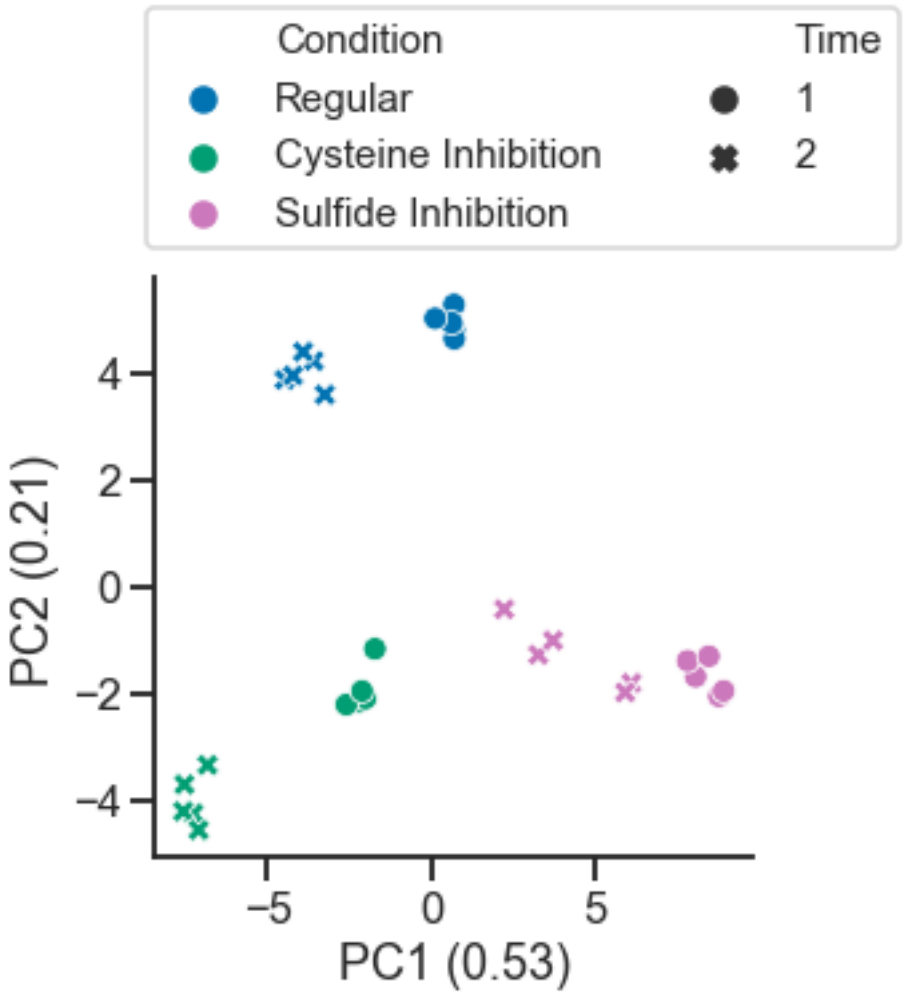
13 Supplementary Figure 4D: PCA Analysis with features mapping to metabolites

[76]:
```
colors=sns.color_palette('colorblind')
cols = ['Cys_1', 'Cys_1', 'Cys_1', 'Cys_1', 'Cys_1', 'Cys_2', 'Cys_2', 'Cys_2',
       'Cys_2', 'Cys_2', 'Reg_1', 'Reg_1', 'Reg_1', 'Reg_1', 'Reg_1',
       'Reg_2', 'Reg_2', 'Reg_2', 'Reg_2', 'Reg_2', 'Sulf_1', 'Sulf_1',
       'Sulf_1', 'Sulf_1', 'Sulf_1', 'Sulf_2', 'Sulf_2', 'Sulf_2',
       'Sulf_2', 'Sulf_2']
cols = [(x.split('_')[0],x.split('_')[1]) for x in cols]
temp = logarea.copy()
temp.columns = pd.MultiIndex.from_tuples(cols)
temp=temp[['Reg','Cys','Sulf']]
logarea2 = temp
```

```
pca_data =temp.loc[idxs,:].copy().T
pca_data=pca_data.apply(zscore)
pca = PCA(n_components=2)
color=sns.color_palette('colorblind')
c=[colors[0],colors[2],colors[4]]
pca_coord = pca.fit_transform(pca_data)
pca_coord= pd.DataFrame(pca_coord,index=pca_data.index,columns=['PC1','PC2'])
pca_coord = pca_coord.reset_index()
pca_coord.columns=['Condition','Time','PC1','PC2']
labels = {'Cys':'Cysteine Inhibition','Reg':'Regular','Sulf':'Sulfide␣
 ↪Inhibition'}
pca_coord['Condition']=pca_coord['Condition'].map(labels)
print('Explained Variance... PC1:%s, PC2:%s'%tuple(pca.
 ↪explained_variance_ratio_))
loadings = pd.DataFrame(pca.components_,index=['PC1','PC2'],columns=pca_data.
 ↪columns)

plt.figure(figsize=(4,4))
sns.
 ↪scatterplot(x='PC1',y='PC2',hue='Condition',style='Time',data=pca_coord,palette=c)
plt.legend(bbox_to_anchor=(-.05,1.5),ncol=2,loc=2,fontsize=15)
plt.xlabel('PC1 (%s)'%(pca.explained_variance_ratio_[0].round(2)))
plt.ylabel('PC2 (%s)'%(pca.explained_variance_ratio_[1].round(2)))
#plt.savefig('Pred_Metabolites_PCA_All.pdf',bbox_inches='tight')
```

```
Explained Variance… PC1:0.5316343239426855, PC2:0.21420861404263722
```

[76]:
```
Text(0, 0.5, 'PC2 (0.21)')
```



14 Obtain feature pathway mapping

[155]:
```
idx=pathway.index[:282]
feature_idx=[]
pathmap={}
for i in idx:
    idx_set=pathway.loc[i,:]['overlap_input(featureidx)']
    if type(idx_set)!=type(''):
        continue
    path =pathway.loc[i,:]['pathway']
    for ID in idx_set.split(';'):
        for j in ID.split(','):
            if int(j) not in feature_idx:
                feature_idx.append(int(j))
            if int(j) not in pathmap:
```

```
                pathmap[int(j)]=[path]
            elif path not in pathmap[int(j)]:
                pathmap[int(j)].append(path)

pathmap
```

[155]:
```
{68: ['isoleucine biosynthesis I (from threonine)',
  'methionine biosynthesis I',
  'cysteine biosynthesis/homocysteine degradation'],
 234: ['isoleucine biosynthesis I (from threonine)',
  'valine biosynthesis',
  '2-oxopentenoate degradation',
  'phosphopantothenate biosynthesis I',
  'androstenedione degradation'],
 117: ['isoleucine biosynthesis I (from threonine)',
  'valine biosynthesis',
  'incomplete reductive TCA cycle',
  '2-oxopentenoate degradation',
  'respiration (anaerobic)',
  'mixed acid fermentation',
  'methylerythritol phosphate pathway II',
  'gentisate degradation',
  'lysine biosynthesis I',
  'alanine biosynthesis I',
  '4-aminobenzoate biosynthesis',
  'L-ascorbate biosynthesis IV',
  'thiazole biosynthesis I (E. coli)',
  'tryptophan biosynthesis',
  "pyridoxal 5'-phosphate biosynthesis I",
  'gluconeogenesis I',
  'androstenedione degradation',
  'streptomycin biosynthesis',
  'alanine degradation IV',
  'pyruvate fermentation to acetate II',
  'D-fructuronate degradation',
  'pyruvate fermentation to lactate',
  '<i>N</i>-acetylneuraminate and <i>N</i>-acetylmannosamine degradation',
  'pyruvate fermentation to ethanol III',
  'glycolysis II (from fructose 6-phosphate)',
  'glycolysis III (from glucose)',
  'L-serine degradation',
  'L-cysteine degradation II',
  'tryptophan degradation II (via pyruvate)',
  'pyruvate decarboxylation to acetyl CoA',
  'thiazole biosynthesis II (Bacillus)'],
 241: ['isoleucine biosynthesis I (from threonine)',
  'valine biosynthesis',
```

```
 'incomplete reductive TCA cycle',
 '2-oxopentenoate degradation',
 'respiration (anaerobic)',
 'glyoxylate cycle',
 'mixed acid fermentation',
 'methylerythritol phosphate pathway II',
 'gentisate degradation',
 'TCA cycle III (helicobacter)',
 'lysine biosynthesis I',
 'TCA cycle IV (2-oxoglutarate decarboxylase)',
 'TCA cycle V (2-oxoglutarate:ferredoxin oxidoreductase)',
 'alanine biosynthesis I',
 '4-aminobenzoate biosynthesis',
 'thiazole biosynthesis I (E. coli)',
 'tryptophan biosynthesis',
 "pyridoxal 5'-phosphate biosynthesis I",
 'gluconeogenesis I',
 'androstenedione degradation',
 'streptomycin biosynthesis',
 'alanine degradation IV',
 'pyruvate fermentation to acetate II',
 'D-fructuronate degradation',
 'pyruvate fermentation to lactate',
 '<i>N</i>-acetylneuraminate and <i>N</i>-acetylmannosamine degradation',
 'pyruvate fermentation to ethanol III',
 'glycolysis II (from fructose 6-phosphate)',
 'glycolysis III (from glucose)',
 'L-serine degradation',
 'L-cysteine degradation II',
 'tryptophan degradation II (via pyruvate)',
 'pyruvate decarboxylation to acetyl CoA',
 'thiazole biosynthesis II (Bacillus)'],
174: ['isoleucine biosynthesis I (from threonine)',
 'valine biosynthesis',
 'incomplete reductive TCA cycle',
 '2-oxopentenoate degradation',
 'respiration (anaerobic)',
 'mixed acid fermentation',
 'methylerythritol phosphate pathway II',
 'gentisate degradation',
 'lysine biosynthesis I',
 'alanine biosynthesis I',
 '4-aminobenzoate biosynthesis',
 'L-ascorbate biosynthesis IV',
 'thiazole biosynthesis I (E. coli)',
 'tryptophan biosynthesis',
 "pyridoxal 5'-phosphate biosynthesis I",
```

```
 'gluconeogenesis I',
 'androstenedione degradation',
 'streptomycin biosynthesis',
 'alanine degradation IV',
 'pyruvate fermentation to acetate II',
 'D-fructuronate degradation',
 'pyruvate fermentation to lactate',
 '<i>N</i>-acetylneuraminate and <i>N</i>-acetylmannosamine degradation',
 'pyruvate fermentation to ethanol III',
 'glycolysis II (from fructose 6-phosphate)',
 'glycolysis III (from glucose)',
 'L-serine degradation',
 'L-cysteine degradation II',
 'tryptophan degradation II (via pyruvate)',
 'pyruvate decarboxylation to acetyl CoA',
 'thiazole biosynthesis II (Bacillus)'],
197: ['isoleucine biosynthesis I (from threonine)',
 'valine biosynthesis',
 'incomplete reductive TCA cycle',
 '2-oxopentenoate degradation',
 'respiration (anaerobic)',
 'mixed acid fermentation',
 'methylerythritol phosphate pathway II',
 'gentisate degradation',
 'lysine biosynthesis I',
 'alanine biosynthesis I',
 '4-aminobenzoate biosynthesis',
 'L-ascorbate biosynthesis IV',
 'thiazole biosynthesis I (E. coli)',
 'tryptophan biosynthesis',
 "pyridoxal 5'-phosphate biosynthesis I",
 'gluconeogenesis I',
 'androstenedione degradation',
 'streptomycin biosynthesis',
 'alanine degradation IV',
 'pyruvate fermentation to acetate II',
 'D-fructuronate degradation',
 'pyruvate fermentation to lactate',
 '<i>N</i>-acetylneuraminate and <i>N</i>-acetylmannosamine degradation',
 'pyruvate fermentation to ethanol III',
 'glycolysis II (from fructose 6-phosphate)',
 'glycolysis III (from glucose)',
 'L-serine degradation',
 'L-cysteine degradation II',
 'tryptophan degradation II (via pyruvate)',
 'pyruvate decarboxylation to acetyl CoA',
 'thiazole biosynthesis II (Bacillus)'],
```

```
42: ['isoleucine biosynthesis I (from threonine)',
 'valine biosynthesis',
 'incomplete reductive TCA cycle',
 '2-oxopentenoate degradation',
 'respiration (anaerobic)',
 'mixed acid fermentation',
 'methylerythritol phosphate pathway II',
 'gentisate degradation',
 'lysine biosynthesis I',
 'alanine biosynthesis I',
 '4-aminobenzoate biosynthesis',
 'L-ascorbate biosynthesis IV',
 'thiazole biosynthesis I (E. coli)',
 'tryptophan biosynthesis',
 "pyridoxal 5'-phosphate biosynthesis I",
 'gluconeogenesis I',
 'androstenedione degradation',
 'streptomycin biosynthesis',
 'alanine degradation IV',
 'pyruvate fermentation to acetate II',
 'D-fructuronate degradation',
 'pyruvate fermentation to lactate',
 '<i>N</i>-acetylneuraminate and <i>N</i>-acetylmannosamine degradation',
 'pyruvate fermentation to ethanol III',
 'glycolysis II (from fructose 6-phosphate)',
 'glycolysis III (from glucose)',
 'L-serine degradation',
 'L-cysteine degradation II',
 'tryptophan degradation II (via pyruvate)',
 'pyruvate decarboxylation to acetyl CoA',
 'thiazole biosynthesis II (Bacillus)'],
298: ['isoleucine biosynthesis I (from threonine)',
 'valine biosynthesis',
 'incomplete reductive TCA cycle',
 '2-oxopentenoate degradation',
 'respiration (anaerobic)',
 'mixed acid fermentation',
 'methylerythritol phosphate pathway II',
 'gentisate degradation',
 'lysine biosynthesis I',
 'alanine biosynthesis I',
 '4-aminobenzoate biosynthesis',
 'L-ascorbate biosynthesis IV',
 'thiazole biosynthesis I (E. coli)',
 'tryptophan biosynthesis',
 "pyridoxal 5'-phosphate biosynthesis I",
 'gluconeogenesis I',
```

```
 'androstenedione degradation',
 'streptomycin biosynthesis',
 'alanine degradation IV',
 'pyruvate fermentation to acetate II',
 'D-fructuronate degradation',
 'pyruvate fermentation to lactate',
 '<i>N</i>-acetylneuraminate and <i>N</i>-acetylmannosamine degradation',
 'pyruvate fermentation to ethanol III',
 'glycolysis II (from fructose 6-phosphate)',
 'glycolysis III (from glucose)',
 'L-serine degradation',
 'L-cysteine degradation II',
 'tryptophan degradation II (via pyruvate)',
 'pyruvate decarboxylation to acetyl CoA',
 'thiazole biosynthesis II (Bacillus)'],
102: ['isoleucine biosynthesis I (from threonine)',
 'valine biosynthesis',
 'incomplete reductive TCA cycle',
 '2-oxopentenoate degradation',
 'respiration (anaerobic)',
 'mixed acid fermentation',
 'methylerythritol phosphate pathway II',
 'gentisate degradation',
 'lysine biosynthesis I',
 'alanine biosynthesis I',
 '4-aminobenzoate biosynthesis',
 'L-ascorbate biosynthesis IV',
 'thiazole biosynthesis I (E. coli)',
 'tryptophan biosynthesis',
 "pyridoxal 5'-phosphate biosynthesis I",
 'gluconeogenesis I',
 'androstenedione degradation',
 'streptomycin biosynthesis',
 'alanine degradation IV',
 'pyruvate fermentation to acetate II',
 'D-fructuronate degradation',
 'pyruvate fermentation to lactate',
 '<i>N</i>-acetylneuraminate and <i>N</i>-acetylmannosamine degradation',
 'pyruvate fermentation to ethanol III',
 'glycolysis II (from fructose 6-phosphate)',
 'glycolysis III (from glucose)',
 'L-serine degradation',
 'L-cysteine degradation II',
 'tryptophan degradation II (via pyruvate)',
 'pyruvate decarboxylation to acetyl CoA',
 'thiazole biosynthesis II (Bacillus)'],
34: ['isoleucine biosynthesis I (from threonine)',
```

```
 'valine biosynthesis',
 'incomplete reductive TCA cycle',
 '2-oxopentenoate degradation',
 'respiration (anaerobic)',
 'mixed acid fermentation',
 'methylerythritol phosphate pathway II',
 'gentisate degradation',
 'lysine biosynthesis I',
 'alanine biosynthesis I',
 '4-aminobenzoate biosynthesis',
 'L-ascorbate biosynthesis IV',
 'thiazole biosynthesis I (E. coli)',
 'tryptophan biosynthesis',
 "pyridoxal 5'-phosphate biosynthesis I",
 'gluconeogenesis I',
 'androstenedione degradation',
 'streptomycin biosynthesis',
 'alanine degradation IV',
 'pyruvate fermentation to acetate II',
 'D-fructuronate degradation',
 'pyruvate fermentation to lactate',
 '<i>N</i>-acetylneuraminate and <i>N</i>-acetylmannosamine degradation',
 'pyruvate fermentation to ethanol III',
 'glycolysis II (from fructose 6-phosphate)',
 'glycolysis III (from glucose)',
 'L-serine degradation',
 'L-cysteine degradation II',
 'tryptophan degradation II (via pyruvate)',
 'pyruvate decarboxylation to acetyl CoA',
 'thiazole biosynthesis II (Bacillus)'],
213: ['isoleucine biosynthesis I (from threonine)',
 'phosphopantothenate biosynthesis I',
 'lysine fermentation to acetate and butyrate'],
44: ['isoleucine biosynthesis I (from threonine)',
 'cardiolipin biosynthesis I',
 'phosphopantothenate biosynthesis I',
 'glycerol degradation I',
 'triacylglycerol degradation'],
179: ['isoleucine biosynthesis I (from threonine)',
 'leucine biosynthesis',
 'isoleucine degradation I'],
38: ['isoleucine biosynthesis I (from threonine)',
 'valine biosynthesis',
 'phosphopantothenate biosynthesis I',
 'alanine biosynthesis I',
 'leucine biosynthesis',
 'valine degradation I',
```

```
 'isoleucine degradation I'],
100: ['cardiolipin biosynthesis I',
 'incomplete reductive TCA cycle',
 'respiration (anaerobic)',
 'glycerol degradation I',
 'aerobic respiration I (cytochrome c)',
 'glyoxylate cycle',
 'mixed acid fermentation',
 'TCA cycle III (helicobacter)',
 'lysine biosynthesis I',
 'TCA cycle IV (2-oxoglutarate decarboxylase)',
 'TCA cycle V (2-oxoglutarate:ferredoxin oxidoreductase)',
 'methionine biosynthesis I',
 'CDP-diacylglycerol biosynthesis I',
 '4-aminobutyrate degradation II',
 'ketolysis',
 'glycerophosphodiester degradation',
 '4-aminobutyrate degradation III',
 '3-oxoadipate degradation',
 '4-aminobutyrate degradation I'],
303: ['cardiolipin biosynthesis I',
 'glycerol degradation I',
 'CDP-diacylglycerol biosynthesis I',
 'glycerophosphodiester degradation'],
53: ['cardiolipin biosynthesis I',
 'pyrimidine ribonucleosides salvage I',
 'methylerythritol phosphate pathway II',
 'pyrimidine ribonucleotides salvage',
 'purine and pyrimidine metabolism',
 'CMP phosphorylation',
 'coenzyme A biosynthesis',
 'phosphatidylethanolamine biosynthesis I'],
161: ['valine biosynthesis',
 '2-oxopentenoate degradation',
 'catechol degradation to 2-oxopent-4-enoate I',
 'catechol degradation to 2-oxopent-4-enoate II',
 'protocatechuate degradation III (<I>para</I>-cleavage pathway)',
 'xylose degradation I'],
184: ['valine biosynthesis',
 '2-oxopentenoate degradation',
 'threonine degradation III (to methylglyoxal)',
 'xylose degradation I',
 'acrylonitrile degradation I'],
158: ['valine biosynthesis',
 '2-oxopentenoate degradation',
 'catechol degradation to 2-oxopent-4-enoate I',
 'catechol degradation to 2-oxopent-4-enoate II',
```

```
  'protocatechuate degradation III (<I>para</I>-cleavage pathway)',
  'xylose degradation I'],
 220: ['incomplete reductive TCA cycle',
  'respiration (anaerobic)',
  'aerobic respiration I (cytochrome c)',
  'glyoxylate cycle',
  'mixed acid fermentation',
  'gentisate degradation',
  'TCA cycle III (helicobacter)',
  'TCA cycle IV (2-oxoglutarate decarboxylase)',
  'TCA cycle V (2-oxoglutarate:ferredoxin oxidoreductase)',
  'arginine biosynthesis II (acetyl cycle)',
  'gluconeogenesis I',
  "inosine-5'-phosphate biosynthesis II",
  'adenosine ribonucleotides <i>de novo</i> biosynthesis',
  'citrulline-nitric oxide cycle',
  "inosine-5'-phosphate biosynthesis I",
  'urea cycle',
  'arginine biosynthesis IV (archaebacteria)'],
 239: ['catechol degradation to 2-oxopent-4-enoate I',
  "pyridoxal 5'-phosphate salvage pathway",
  'catechol degradation to 2-oxopent-4-enoate II',
  'protocatechuate degradation III (<I>para</I>-cleavage pathway)'],
 27: ['threonine degradation III (to methylglyoxal)',
  'threonine degradation II'],
 289: ['pyrimidine ribonucleosides salvage I',
  'UMP biosynthesis',
  'pyrimidine ribonucleotides salvage',
  'purine and pyrimidine metabolism',
  'UTP and CTP biosynthesis',
  'pseudouridine degradation',
  'pyrimidine nucleobases salvage I'],
 115: ['UDP-<i>N</i>-acetyl-&alpha;-D-mannosaminouronate biosynthesis',
  'UDP-<i>N</i>-acetyl-D-glucosamine biosynthesis I',
  'CMP-pseudaminate biosynthesis',
  'anhydromuropeptides recycling',
  'mycothiol biosynthesis',
  'UDP-<i>N</i>-acetylmuramoyl-pentapeptide biosynthesis II (lysine-
containing)',
  'UDP-<i>N</i>-acetylmuramoyl-pentapeptide biosynthesis III (<i>meso</i>-DAP-
containing)',
  'CMP-<i>N</i>-acetylneuraminate biosynthesis II (bacteria)'],
 296: ['UDP-<i>N</i>-acetyl-&alpha;-D-mannosaminouronate biosynthesis',
  'UDP-<i>N</i>-acetyl-D-glucosamine biosynthesis I',
  'CMP-pseudaminate biosynthesis',
  'anhydromuropeptides recycling',
  'mycothiol biosynthesis',
```

```
  'UDP-<i>N</i>-acetylmuramoyl-pentapeptide biosynthesis II (lysine-
containing)',
  'UDP-<i>N</i>-acetylmuramoyl-pentapeptide biosynthesis III (<i>meso</i>-DAP-
containing)',
  'CMP-<i>N</i>-acetylneuraminate biosynthesis II (bacteria)'],
 110: ['methylerythritol phosphate pathway II'],
 113: ["pyridoxal 5'-phosphate salvage pathway"],
 111: ['biotin biosynthesis from 7-keto-8-aminopelargonate'],
 242: ['biotin biosynthesis from 7-keto-8-aminopelargonate',
  '4-amino-2-methyl-5-diphosphomethylpyrimidine biosynthesis',
  'thiazole biosynthesis I (E. coli)',
  'cyclopropane fatty acid (CFA) biosynthesis',
  'factor 420 biosynthesis',
  'menaquinol-6 biosynthesis',
  '<i>S</i>-adenosyl-L-methionine cycle II',
  'menaquinol-10 biosynthesis',
  'lipoate biosynthesis and incorporation I',
  'menaquinol-11 biosynthesis',
  'S-adenosyl-L-methionine biosynthesis',
  'heme biosynthesis from uroporphyrinogen-III II',
  'cob(II)yrinate <i>a,c</i>-diamide biosynthesis I (early cobalt insertion)',
  'chlorophyllide <i>a</i> biosynthesis III',
  'chlorophyllide <i>a</i> biosynthesis I',
  'methionine degradation I (to homocysteine)',
  'lipoate biosynthesis and incorporation III (Bacillus)',
  'menaquinol-8 biosynthesis',
  'chlorophyllide <i>a</i> biosynthesis II (anaerobic)',
  'menaquinol-9 biosynthesis',
  'cob(II)yrinate <i>a,c</i>-diamide biosynthesis II (late cobalt
incorporation)'],
 39: ['lysine biosynthesis I'],
 21: ['lysine biosynthesis I',
  'arginine biosynthesis II (acetyl cycle)',
  'ornithine biosynthesis'],
 86: ['4-amino-2-methyl-5-diphosphomethylpyrimidine biosynthesis',
  'hydroxymethylpyrimidine salvage',
  'thiamin salvage IV (yeast)'],
 188: ['4-aminobenzoate biosynthesis',
  'tryptophan biosynthesis',
  'tyrosine biosynthesis I',
  'chorismate biosynthesis from 3-dehydroquinate'],
 262: ['methionine biosynthesis I',
  'taxiphyllin bioactivation',
  'thiosulfate disproportionation III (rhodanese)'],
 247: ['UMP biosynthesis'],
 171: ['UDP-<i>N</i>-acetyl-D-glucosamine biosynthesis I',
  'anhydromuropeptides recycling',
```

```
 'streptomycin biosynthesis'],
49: ['arginine biosynthesis II (acetyl cycle)', 'ornithine biosynthesis'],
153: ["pyridoxal 5'-phosphate biosynthesis I"],
96: ["pyridoxal 5'-phosphate biosynthesis I"],
210: ['purine and pyrimidine metabolism',
 'dTMP <i>de novo</i> biosynthesis (mitochondrial)',
 'pyrimidine deoxyribonucleosides salvage',
 'pyrimidine deoxyribonucleotides <i>de novo</i> biosynthesis I',
 'pyrimidine deoxyribonucleotides <i>de novo</i> biosynthesis II',
 'salvage pathways of pyrimidine deoxyribonucleotides',
 '<i>N</i><sup>10</sup>-formyl-tetrahydrofolate biosynthesis',
 'pyrimidine deoxyribonucleotide phosphorylation'],
101: ['CMP-pseudaminate biosynthesis'],
233: ['CMP-pseudaminate biosynthesis'],
67: ['lysine fermentation to acetate and butyrate'],
71: ['lysine fermentation to acetate and butyrate'],
254: ['xanthine and xanthosine salvage',
 "urate biosynthesis/inosine 5'-phosphate degradation",
 'purine ribonucleosides degradation'],
215: ['lysine degradation VI'],
312: ['oleate &beta;-oxidation'],
280: ['dTDP-L-rhamnose biosynthesis I'],
79: ['sulfate activation for sulfonation'],
105: ['palmitate biosynthesis II (bacteria and plants)']}
```

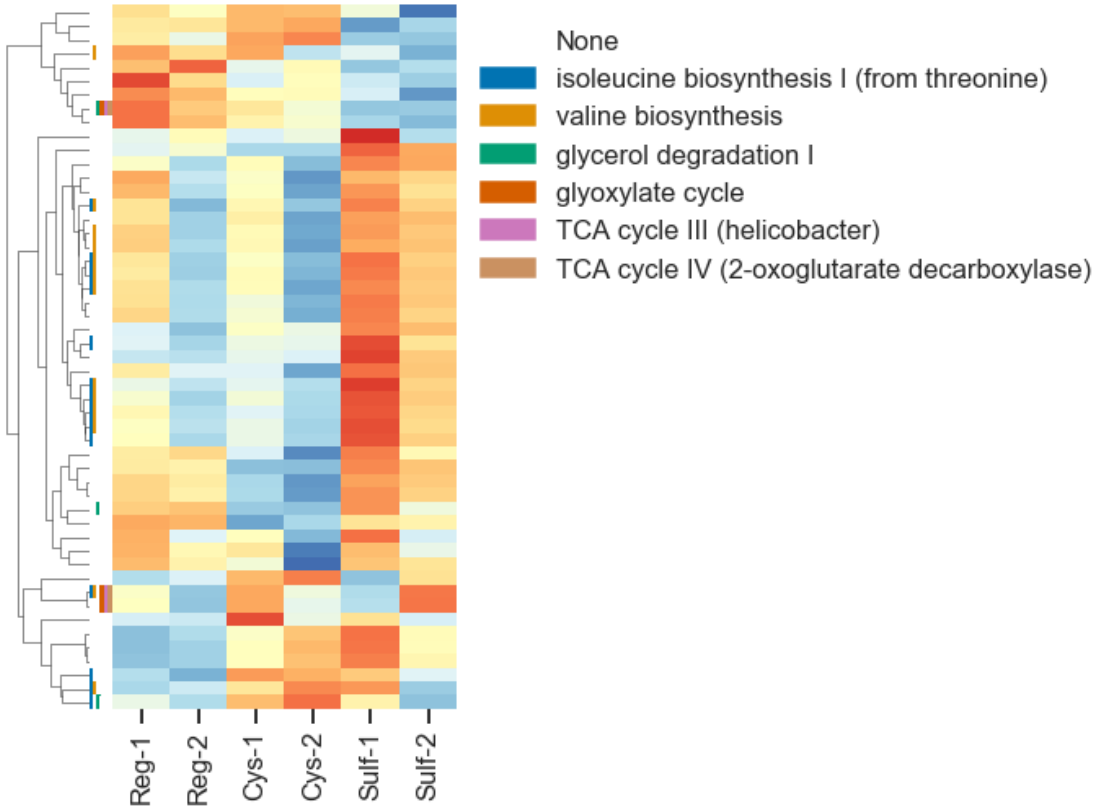
[ ]:
```
def kmeans_cluster(df,k):
    X = df.values
    km = KMeans(n_clusters=k, random_state=0)
    return km.fit_predict(X)
```

15 Figure 3A: Heatmap of feature z-scores for features mapping to metabolites

[18]:
```
df = pca_data.T.groupby(level=[0,1],axis=1).mean().loc[idxs,:].copy()
df=df[['Reg','Cys','Sulf']]
paths=['isoleucine biosynthesis I (from threonine)','valine␣
 ↪biosynthesis','glycerol degradation I','glyoxylate cycle','TCA cycle III␣
 ↪(helicobacter)','TCA cycle IV (2-oxoglutarate decarboxylase)']
colors = sns.color_palette('colorblind')
i=0
path_clr = {'None':(1,1,1)}
row_clrs=[]
for p in paths:
    path_clr[p]=colors[i]
    row_clrs.append(pd.Series(index=df.index,data=np.nan))
    for idx in df.index:
```

```
        if p in pathmap[idx]:
            row_clrs[i][idx]=p
        else:
            row_clrs[i][idx]='None'
    row_clrs[i]=row_clrs[i].map(path_clr)

    i+=1

#If using plt.cm.tab20 then colors[i] must be changed to colors(i)
handles = [mpatches.Patch(color=path_clr[i], label=i) for i in path_clr.keys()]

#Creat heatmap
clust = sns.
 ↪clustermap(df,cmap='RdYlBu',yticklabels='',figsize=(5,10),col_cluster=False,vmax=-2.
 ↪5,vmin=2.5,row_colors=row_clrs)
clust.ax_heatmap.set(ylabel='',xlabel='')
clust.cax.set_visible(False)

clust.ax_heatmap.
 ↪legend(bbox_to_anchor=(1,1),loc=2,handles=handles,frameon=False)
#plt.savefig('Pred-Metabolite-Heatmap.png',bbox_inches='tight',dpi=500)
```

[18]: <matplotlib.legend.Legend at 0x1a241d7e48>



16 Figure 4B: Putative features abundances in BCAA

[19]:
```
names=['CPD-15056','1-KETO-2-METHYLVALERATE','2-KETO-3-METHYL-VALERATE','2-ACETO-2-HYDROXY-BUT
index=[]
name_dict={}
for name in names:
    if type(cmp_dict[name]) == type([]):
        idx=cmp_dict[name]
    else:
        idx=[cmp_dict[name]]

    index+=idx
    for i in idx:
        if i not in name_dict:
            name_dict[i]=name
```

```
        else:
            name_dict[i]=name_dict[i] +'/'+name
name_dict
```

[19]:
```
{68: 'CPD-15056',
 213: '1-KETO-2-METHYLVALERATE',
 44: '1-KETO-2-METHYLVALERATE/GLYCEROL',
 179: '2-KETO-3-METHYL-VALERATE',
 38: '2-KETO-3-METHYL-VALERATE/2-KETO-ISOVALERATE',
 234: '2-ACETO-2-HYDROXY-BUTYRATE/2-ACETO-LACTATE',
 161: '2-ACETO-LACTATE',
 184: '2-ACETO-LACTATE',
 158: '2-ACETO-LACTATE',
 220: 'MAL',
 241: 'MAL',
 100: 'GLYCEROL-3P',
 303: 'GLYCEROL-3P'}
```

[157]:
```
multi=[]
for cond in ['cys','reg','sulf']:
    for t in ['T1','T2']:
        for rep in range(1,6):
            multi.append((cond,t,rep))

relabund=(np.exp(logarea)-1).copy()
relabund.columns=pd.MultiIndex.from_tuples(multi)
relabund=relabund/relabund.sum(axis=0)
relabund.sum(axis=0)
relabund
```

[157]:

	cys				\
	T1				
	1	2	3	4	5
featureidx					
2	0.000003	1.534291e-06	2.482685e-06	0.000003	0.000005
3	0.000005	3.164120e-06	6.018955e-06	0.000005	0.000010
4	0.000002	9.313779e-07	1.868791e-06	0.000002	0.000004
5	0.000002	2.161251e-06	3.400159e-06	0.000004	0.000006
6	0.000001	7.828048e-07	1.299537e-06	0.000001	0.000002
…	…	…	…	…	…
29983	0.000009	6.902365e-06	2.084933e-06	0.000004	0.000008
29984	0.000008	6.814050e-06	1.628057e-06	0.000004	0.000007
29985	0.000021	5.139379e-07	1.669532e-05	0.000026	0.000028
29986	0.000014	1.086642e-05	1.330217e-06	0.000003	0.000009
29987	0.000005	4.113982e-06	9.581834e-07	0.000002	0.000005

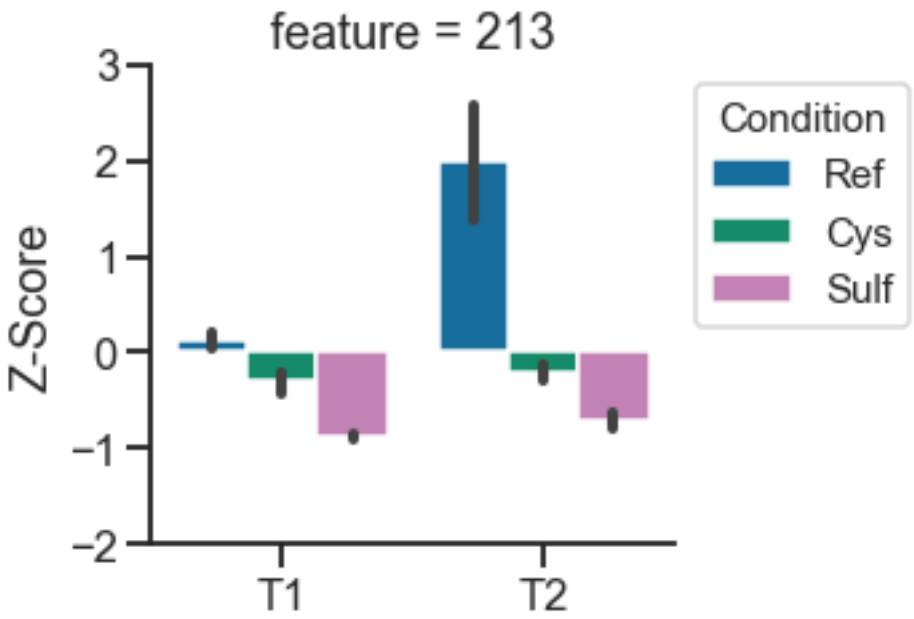
\

```
                    T2
                     1             2             3             4
featureidx
2           4.933268e-08  2.033698e-08  2.201864e-08  2.680995e-08
3           7.142940e-07  7.961235e-07  8.298417e-07  8.040248e-07
4           2.694299e-08  9.586718e-09  5.254944e-08  3.257441e-08
5           1.839371e-07  1.307271e-07  1.542928e-07  2.095020e-07
6           1.333526e-07  1.439539e-07  1.327211e-07  1.715190e-07
…                    …             …             …             …
29983       5.988915e-06  1.520856e-05  2.217725e-06  3.786984e-06
29984       5.200672e-06  1.541116e-05  1.828297e-06  3.430294e-06
29985       2.362840e-05  9.235334e-06  1.443436e-05  2.176989e-05
29986       5.687856e-06  2.549465e-05  1.635998e-06  3.668759e-06
29987       3.036996e-06  8.714433e-06  8.172636e-07  1.693315e-06

                          …          sulf                            \
                          …            T1
                     5    …             1             2             3
featureidx                …
2           7.573280e-08  …  3.746099e-07  7.089190e-07  1.428266e-06
3           8.328074e-07  …  1.300160e-06  1.676210e-06  3.362990e-06
4           1.207442e-08  …  1.872636e-07  4.722305e-07  1.119212e-06
5           2.060366e-07  …  6.861787e-07  1.014798e-06  1.863191e-06
6           1.295445e-07  …  2.969920e-07  3.739778e-07  5.865295e-07
…                    …  …           …             …             …
29983       1.342991e-05  …  9.429891e-06  4.597117e-06  4.962160e-06
29984       1.315275e-05  …  8.450384e-06  3.976927e-06  4.409204e-06
29985       3.881321e-05  …  4.826891e-06  2.511047e-05  2.450187e-05
29986       2.108254e-05  …  1.522642e-05  3.808118e-06  5.024457e-06
29987       7.831434e-06  …  4.933397e-06  2.087852e-06  2.772300e-06

                                                                    \
                                          T2
                     4             5         1         2         3
featureidx
2           1.146211e-06  1.661544e-06  0.000005  0.000005  0.000004
3           2.933160e-06  3.578576e-06  0.000008  0.000009  0.000009
4           1.019705e-06  1.165723e-06  0.000004  0.000003  0.000003
5           1.705198e-06  2.091126e-06  0.000006  0.000006  0.000006
6           7.060791e-07  8.142010e-07  0.000002  0.000002  0.000002
…                    …             …       …         …         …
29983       3.894884e-06  7.946109e-06  0.000006  0.000004  0.000003
29984       2.761636e-06  7.664362e-06  0.000007  0.000002  0.000003
29985       1.769551e-05  2.161258e-05  0.000021  0.000022  0.000021
29986       2.478806e-06  9.763429e-06  0.000009  0.000002  0.000002
29987       1.735353e-06  4.681973e-06  0.000004  0.000001  0.000001
```

```
                    4         5
featureidx
2           0.000006  0.000008
3           0.000010  0.000014
4           0.000004  0.000006
5           0.000007  0.000010
6           0.000002  0.000003
…                  …         …
29983       0.000007  0.000014
29984       0.000005  0.000014
29985       0.000021  0.000012
29986       0.000007  0.000024
29987       0.000003  0.000008

[28508 rows x 30 columns]
```

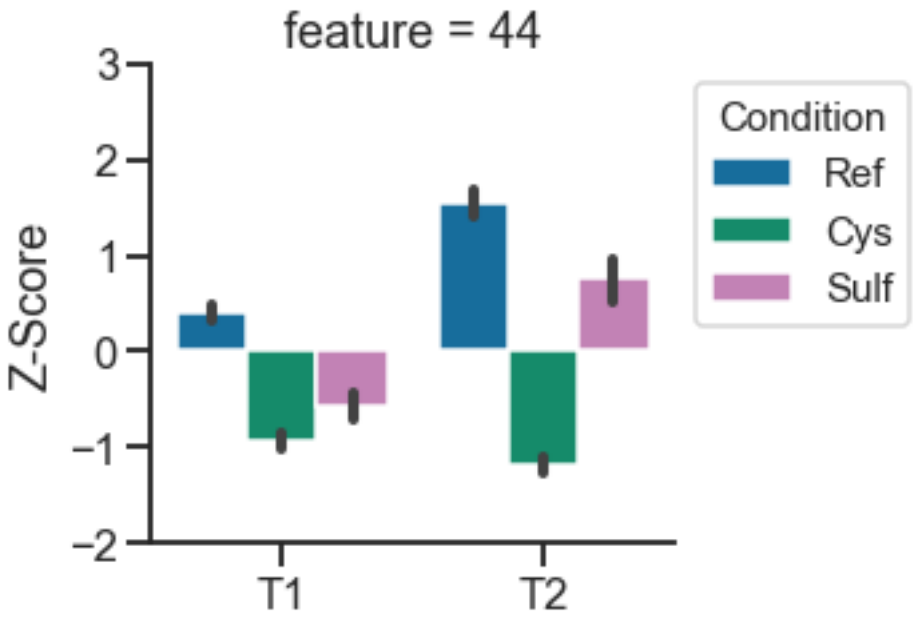
[21]:
```
temp =relabund.T.apply(zscore).T.loc[index,].copy()
df = temp.unstack()
df = df.reset_index()
df['name'] = df['featureidx'].map(name_dict)
df.columns=['cond','t','rep','feature','val','name']
df['cond']=df['cond'].map({'reg':'Ref','cys':'Cys','sulf':'Sulf'})
c = sns.color_palette('colorblind')
c=[c[0],c[2],c[4]]
for name,df2 in df.groupby(by=['name']):
    print(name)
    g = sns.catplot(x="t", y="val",
                hue="cond", col="feature",hue_order=['Ref','Cys','Sulf'],
                data=df2,␣
 ↪kind="bar",palette=c,legend=False,sharey=False,height=4)
    g.axes[0][0].set_ylabel('Z-Score')
    for ax in g.axes[0]:
        ax.set_xlabel('')
        ax.set_yticks([-2,-1,0,1,2,3])
    leg=plt.legend(bbox_to_anchor=(1,1),loc=2,title='Condition',fontsize=15)
    leg.get_title().set_fontsize('15')
    #plt.savefig('%s-box.pdf'%('+'.join(name.split('/'))),bbox_inches='tight')
    plt.show()
    plt.clf()
```

```
1-KETO-2-METHYLVALERATE
```



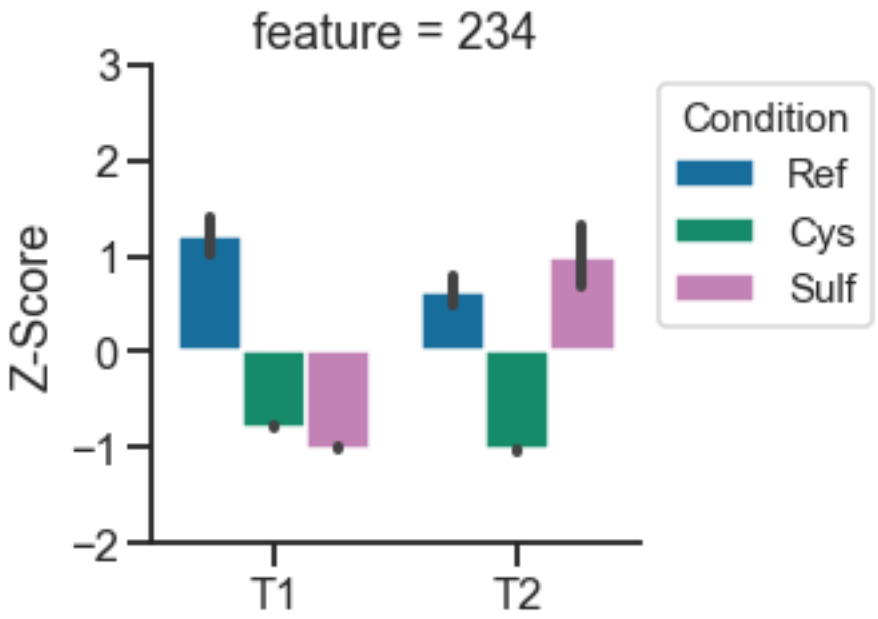
1-KETO-2-METHYLVALERATE/GLYCEROL

<Figure size 432x288 with 0 Axes>



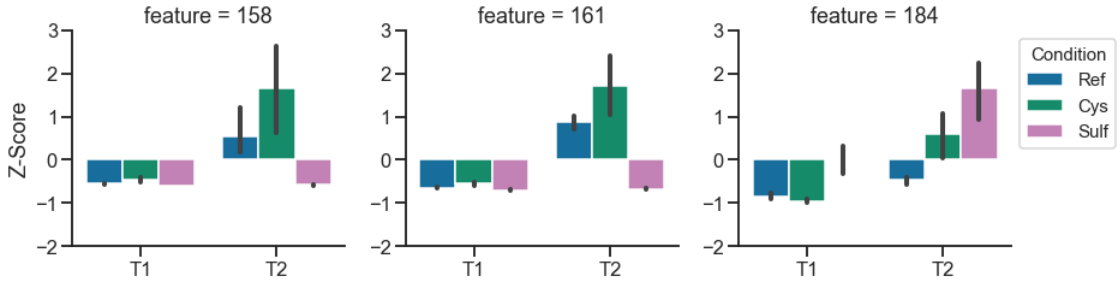
2-ACETO-2-HYDROXY-BUTYRATE/2-ACETO-LACTATE

<Figure size 432x288 with 0 Axes>



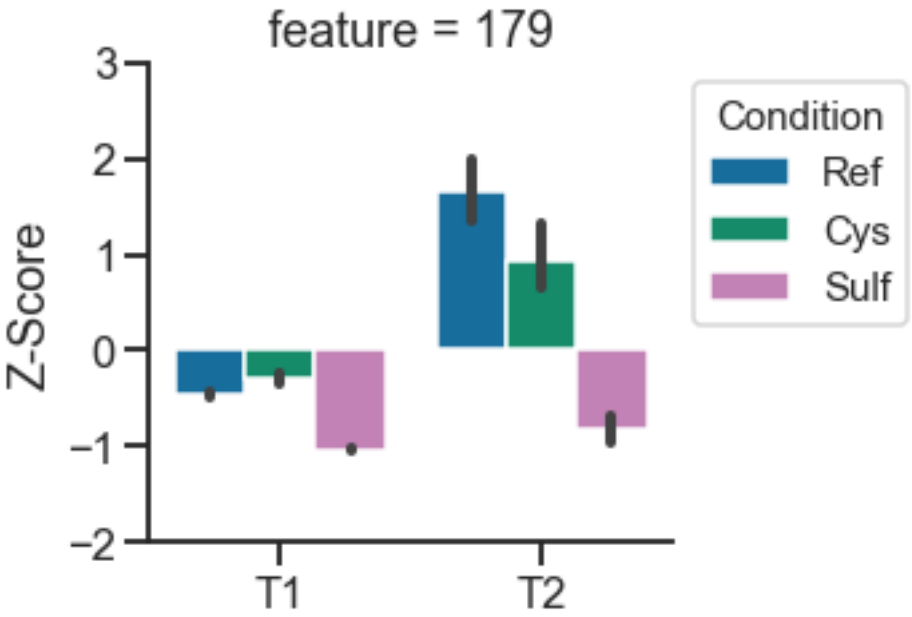
2-ACETO-LACTATE

<Figure size 432x288 with 0 Axes>



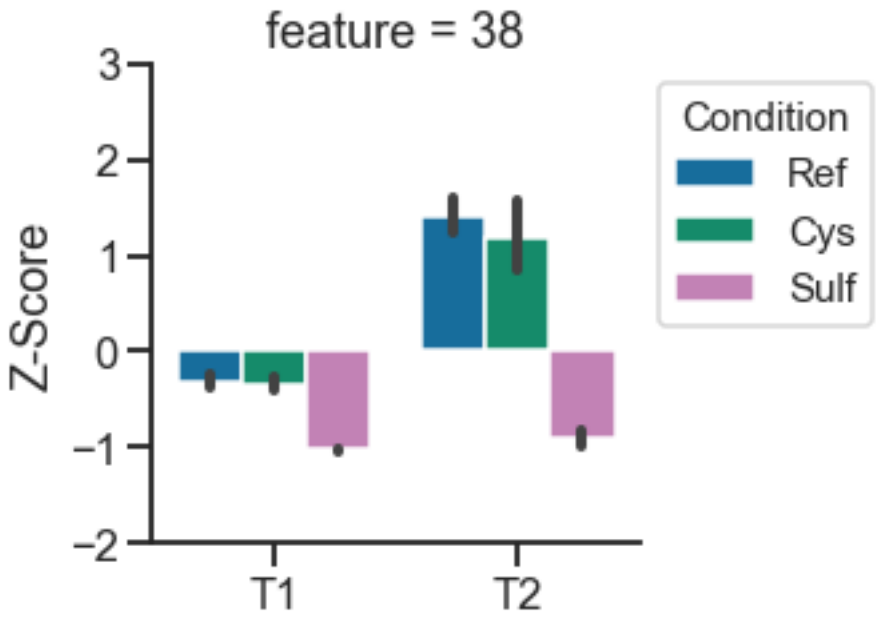
2-KETO-3-METHYL-VALERATE

<Figure size 432x288 with 0 Axes>



2-KETO-3-METHYL-VALERATE/2-KETO-ISOVALERATE

<Figure size 432x288 with 0 Axes>



CPD-15056

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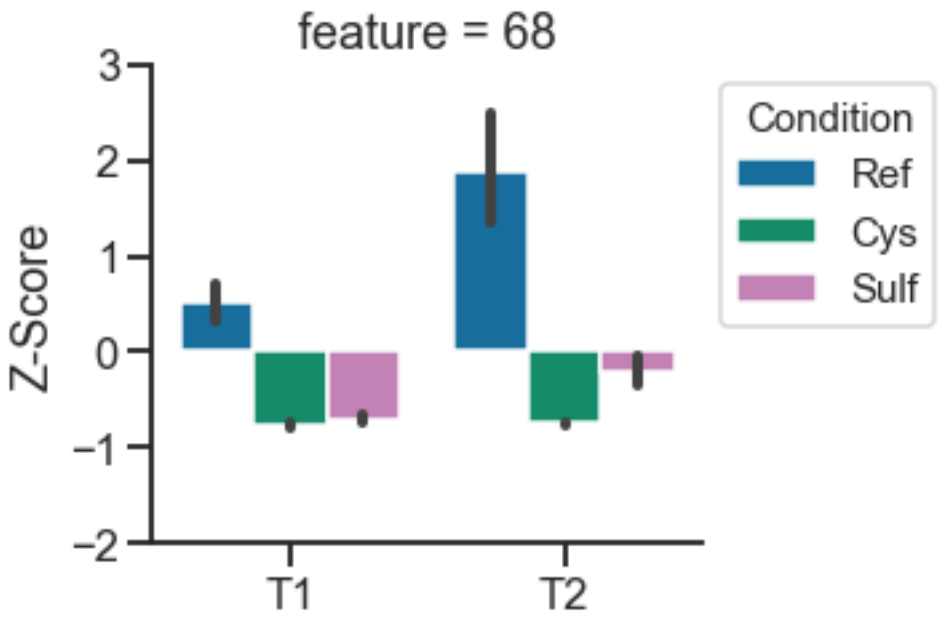

GLYCEROL-3P

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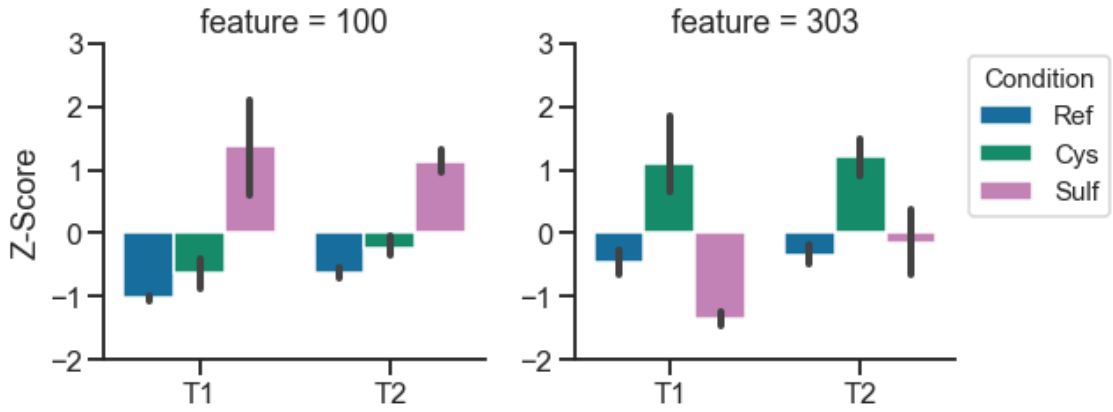
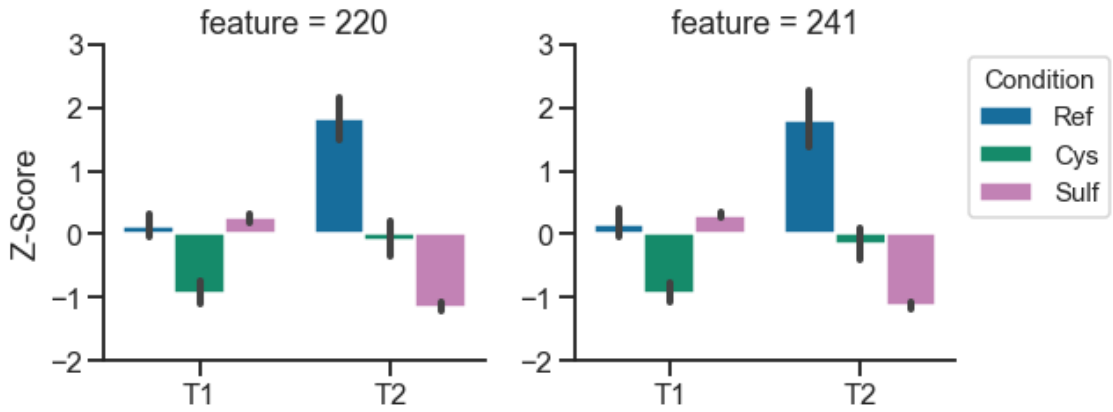


MAL

<Figure size 432x288 with 0 Axes>



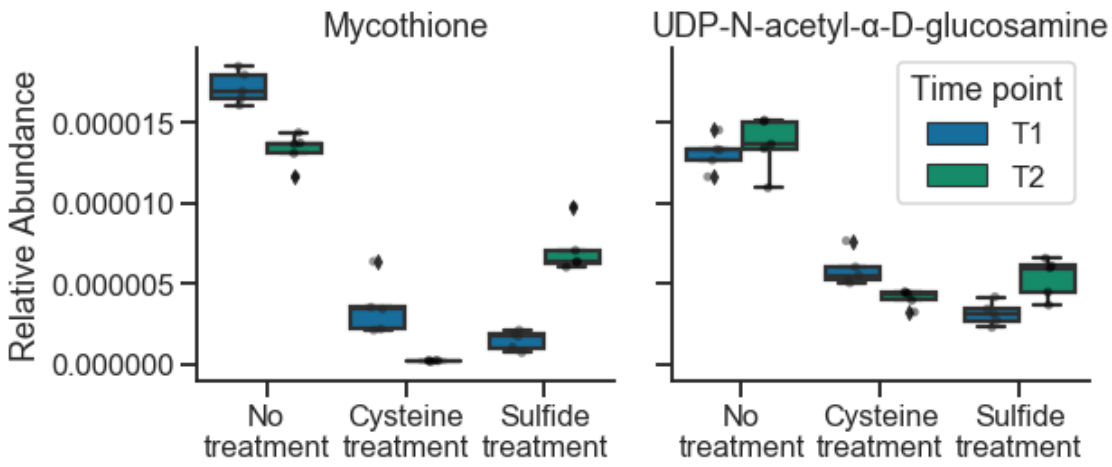

```
<Figure size 432x288 with 0 Axes>
```

17 Supplementary Figure 5: Mycothiol

[166]:
```
df=pd.DataFrame(relabund.loc[[5,115, 296],:])
df=df.T.reset_index()
df.columns=['cond','time','rep','5','115','296']
fig,(ax1,ax2)=plt.subplots(1,2,figsize=(9,4),sharey=True)
c=sns.color_palette('colorblind')

g=sns.
 ↪boxplot(x='cond',y='5',hue='time',data=df,palette=[c[0],c[2],c[4]],order=['reg','cys','sulf
sns.
 ↪stripplot(x='cond',y='5',hue='time',data=df,dodge=True,color='black',alpha=0.
 ↪5,order=['reg','cys','sulf'],ax=ax1)
g.set_ylabel('Relative Abundance')
g.set_xticklabels(['No\ntreatment','Cysteine\ntreatment','Sulfide\ntreatment'])
g.set_title('Mycothione')
g.set_xlabel('')
g.legend().set_visible(False)
g=sns.
 ↪boxplot(x='cond',y='296',hue='time',data=df,palette=[c[0],c[2],c[4]],order=['reg','cys','su
sns.
 ↪stripplot(x='cond',y='296',hue='time',data=df,dodge=True,color='black',alpha=0.
 ↪5,order=['reg','cys','sulf'],ax=ax2)
g.set_ylabel('')
g.set_xlabel('')
handles,labels=g.get_legend_handles_labels()
g.legend(handles[:3],['T1','T2'],bbox_to_anchor=(1,1),title='Time point')
g.set_xticklabels(['No\ntreatment','Cysteine\ntreatment','Sulfide\ntreatment'])
```

```
g.set_title('UDP-N-acetyl- -D-glucosamine')
fig.tight_layout()
plt.savefig('Mycothione-UPD-Relabund.pdf',bbox_inches='tight')
```



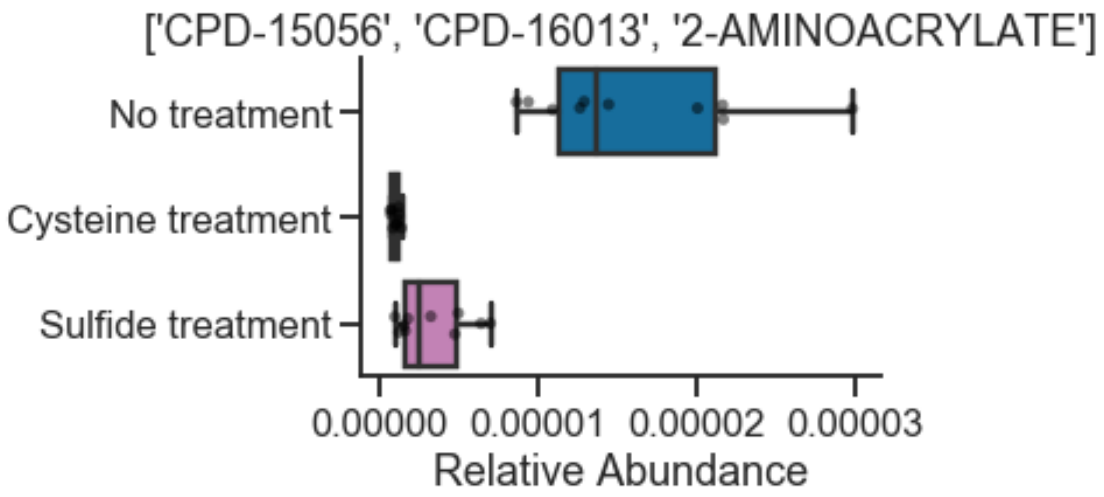
18 Supplementary Figure 4 B

[ ]:
```
data=np.exp(logarea2)-1
data=data/data.sum()
cys_pvals=[]
sulf_pvals=[]
cys_sulf_pvals=[]
c=sns.color_palette('colorblind')
for i in idxs:
    df=data.loc[i].reset_index()
    plt.figure(figsize=(4,2.5))
    g=sns.boxplot(y='level_0',x=i,data=df)
    g=sns.
↪boxplot(y='level_0',x=i,data=df,palette=[c[0],c[2],c[4]],order=['Reg','Cys','Sulf'])
    sns.stripplot(y='level_0',x=i,data=df,color='black',alpha=0.
↪5,order=['Reg','Cys','Sulf'])

    g.set_ylabel('')
    g.set_xlabel('Relative Abundance')
    #g.set_xlim(-3.5,3.5)
    g.set_yticklabels(['No treatment','Cysteine treatment', 'Sulfide␣
↪treatment'])
    reg=df[df['level_0']=='Reg'][i].values
    cys=df[df['level_0']=='Cys'][i].values
    sulf=df[df['level_0']=='Sulf'][i].values
```

```
    cys_pval=ttest_ind(reg,cys,equal_var=False).pvalue
    sulf_pval=ttest_ind(reg,sulf,equal_var=False).pvalue
    cys_sulf_pval=ttest_ind(cys,sulf,equal_var=False).pvalue
    cys_pvals.append(cys_pval)
    sulf_pvals.append(sulf_pval)
    cys_sulf_pvals.append(cys_sulf_pval)
    g.set_title(rev_cmp_dict[i])
    print(i)
    #plt.savefig('metabolomics-feat-%s-boxplots.pdf'%(i),bbox_inches='tight')
    plt.show()
    plt.clf()
cys_pvals=multipletests(cys_pvals, method='fdr_bh',alpha=0.01)[:2][1]
sulf_pvals=multipletests(sulf_pvals, method='fdr_bh',alpha=0.01)[:2][1]
cys_sulf_pvals=multipletests(cys_sulf_pvals, method='fdr_bh',alpha=0.01)[:2][1]
```

68



234

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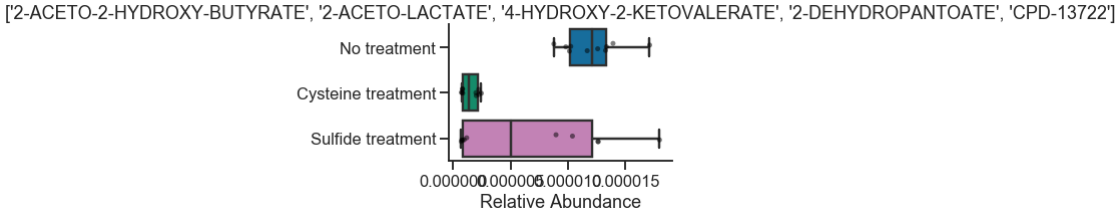

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117
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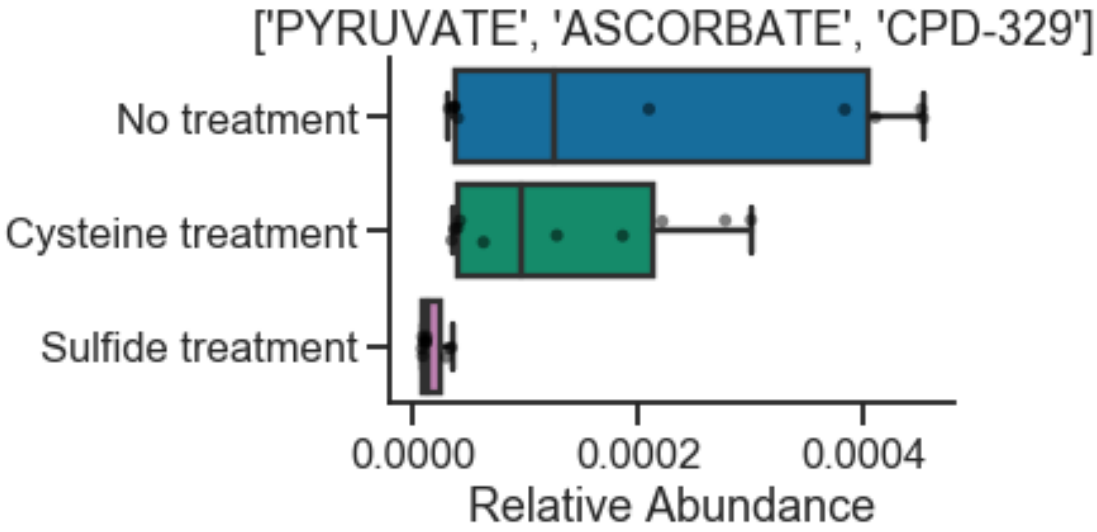


```
241
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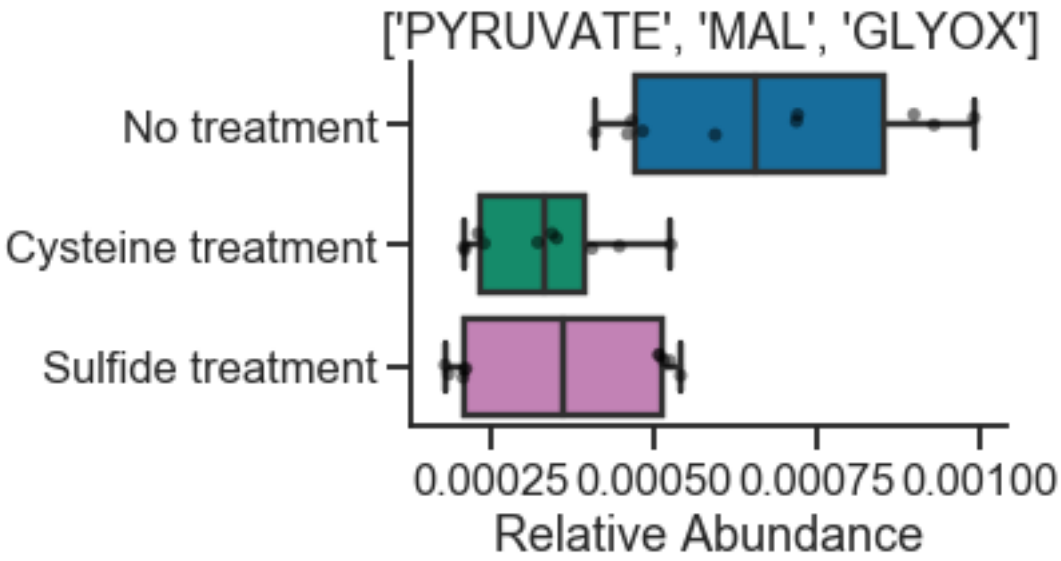


```
174
```

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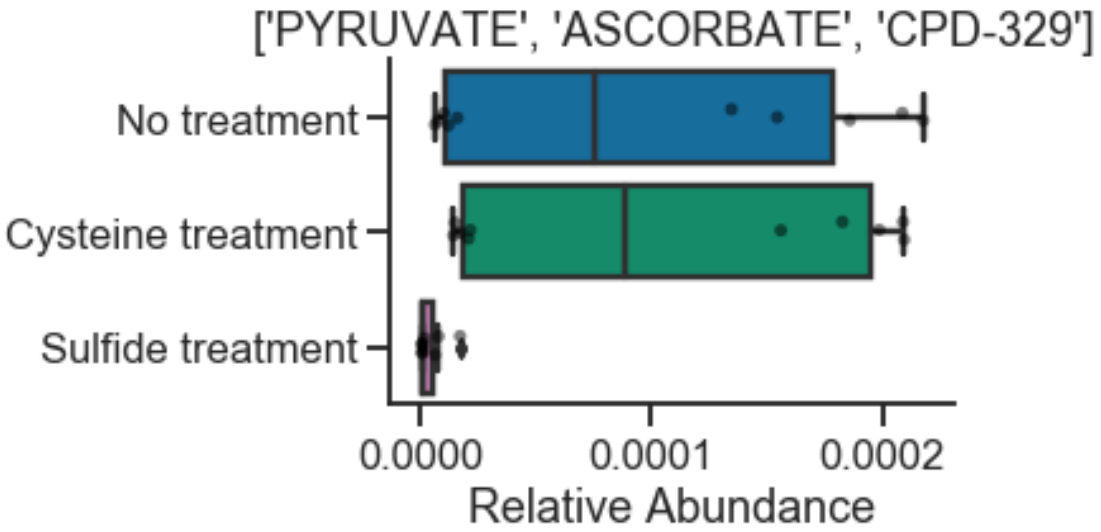

197

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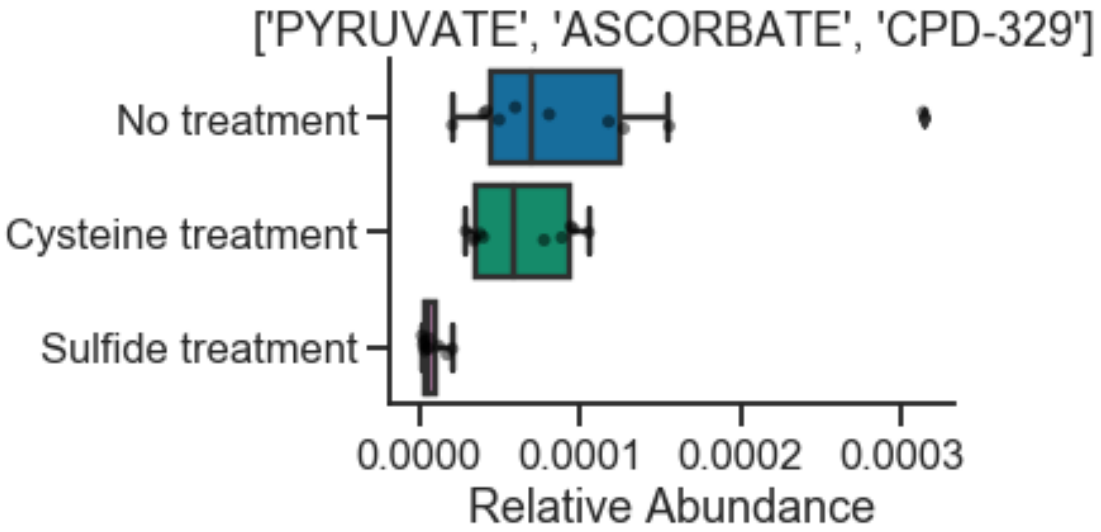

42

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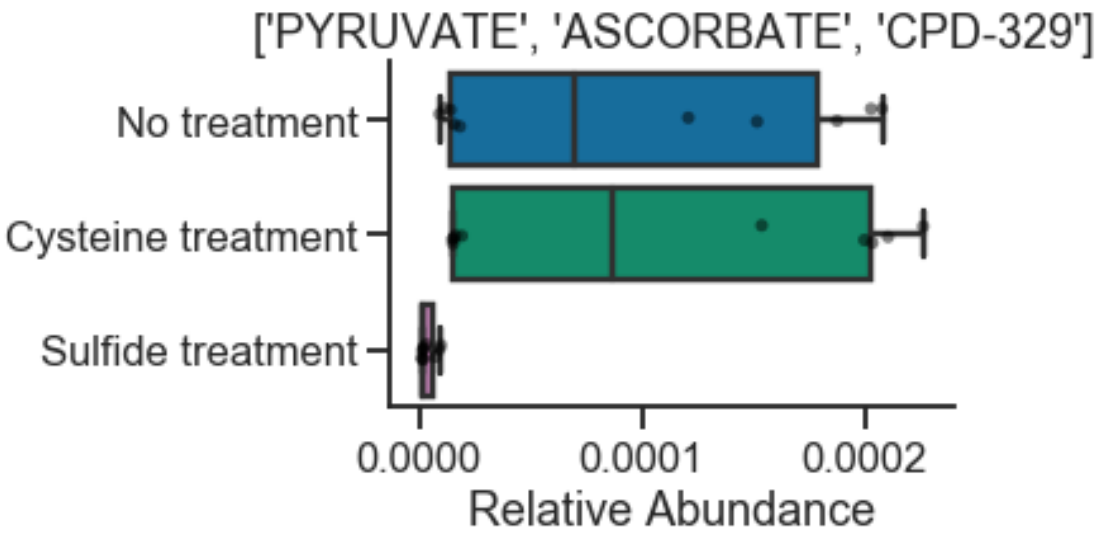

298

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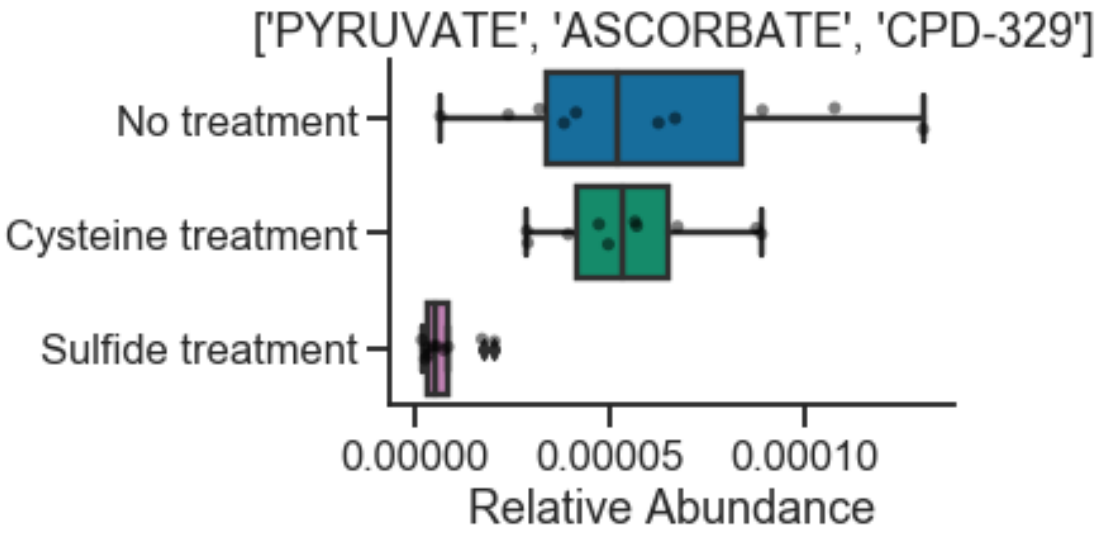

102

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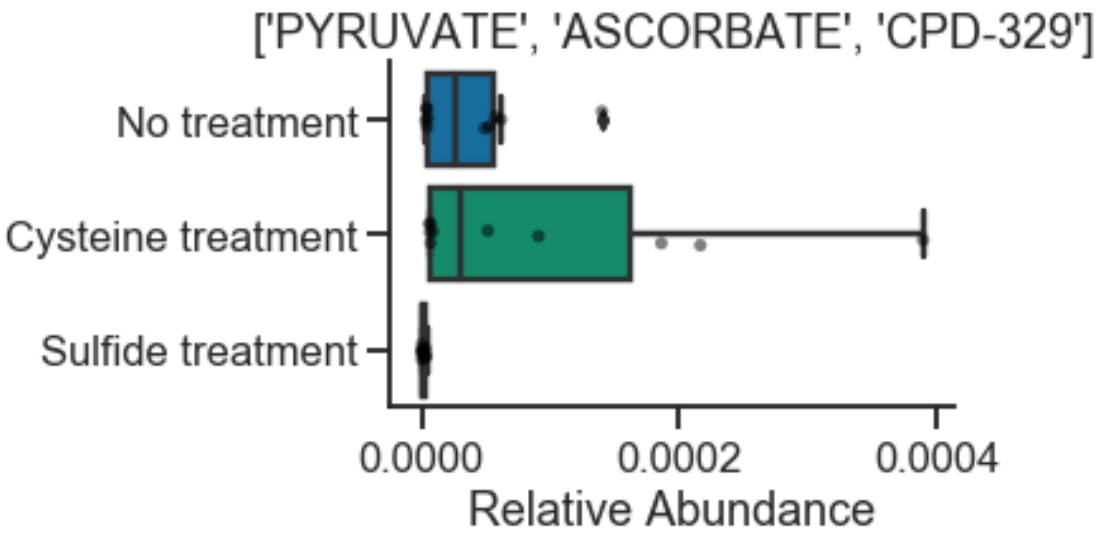


```
34
```

```
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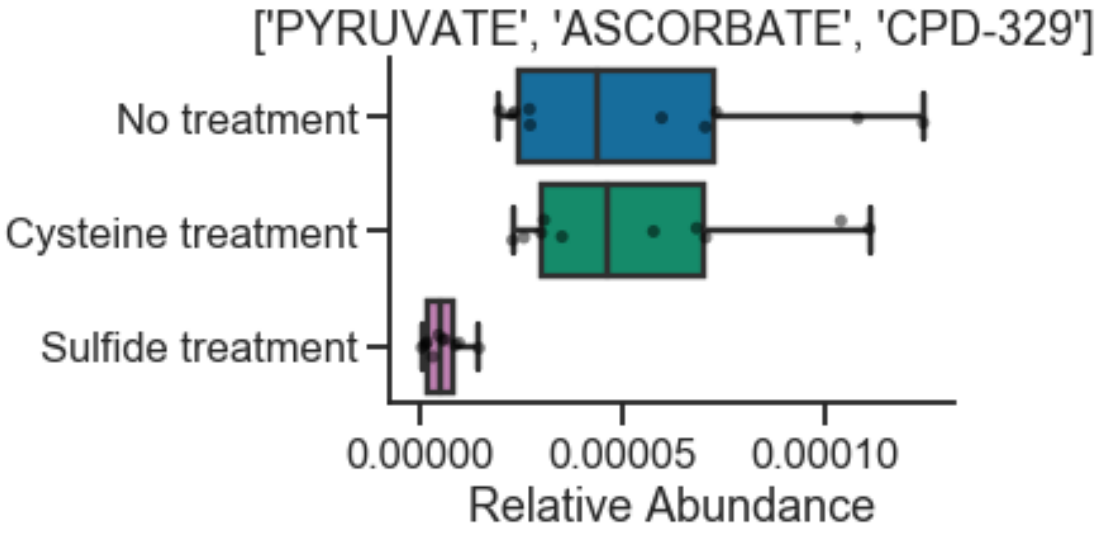


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213
```

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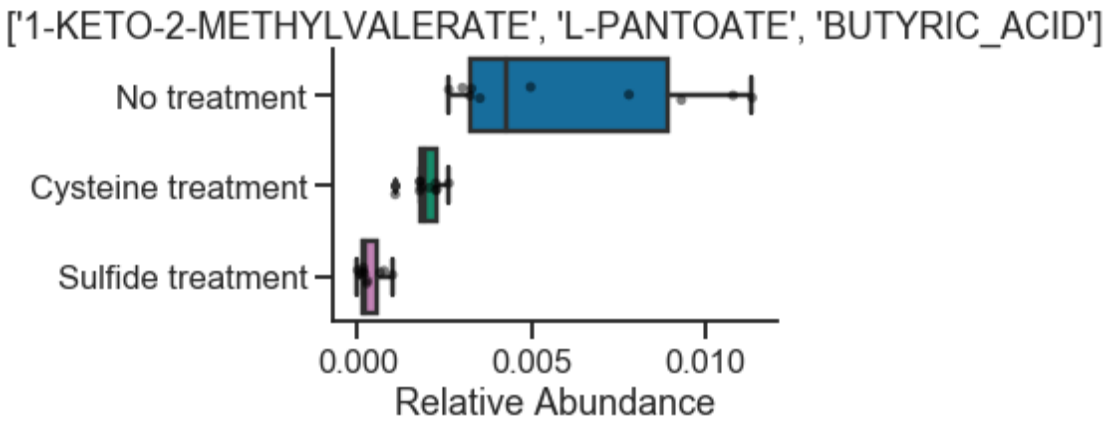


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44
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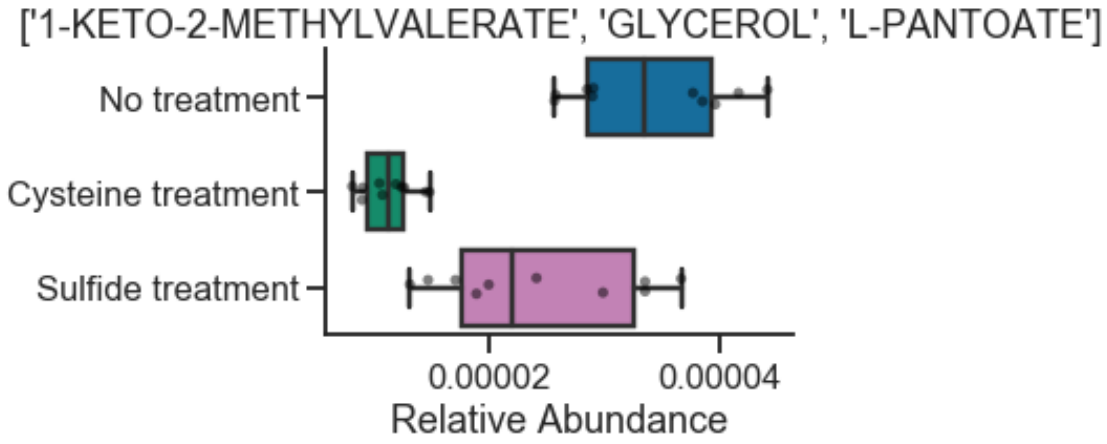


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179
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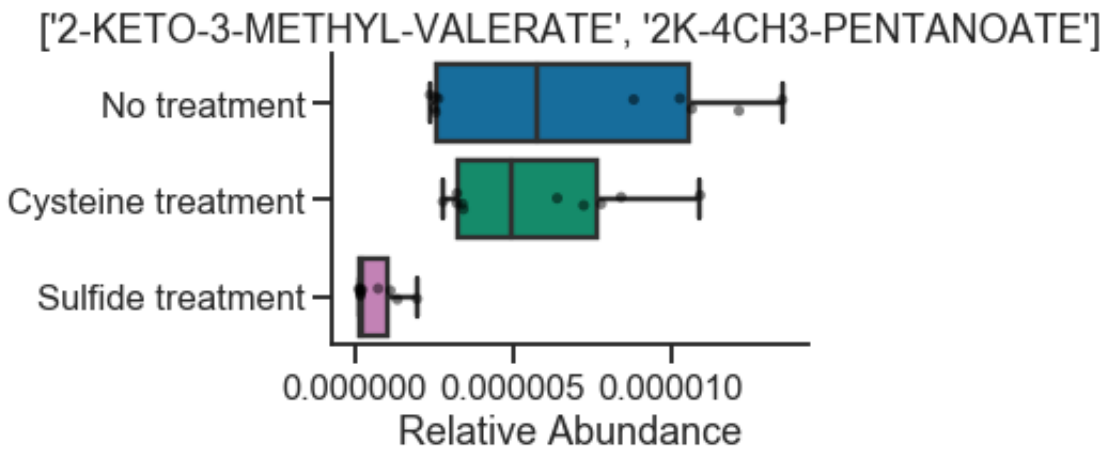

38

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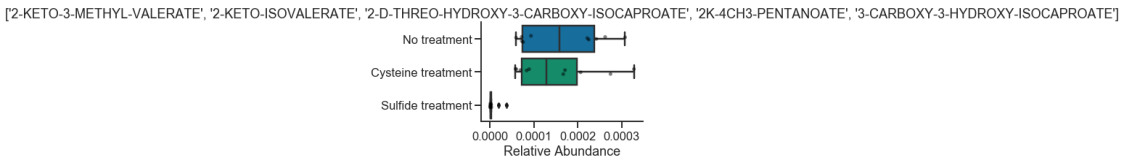

100

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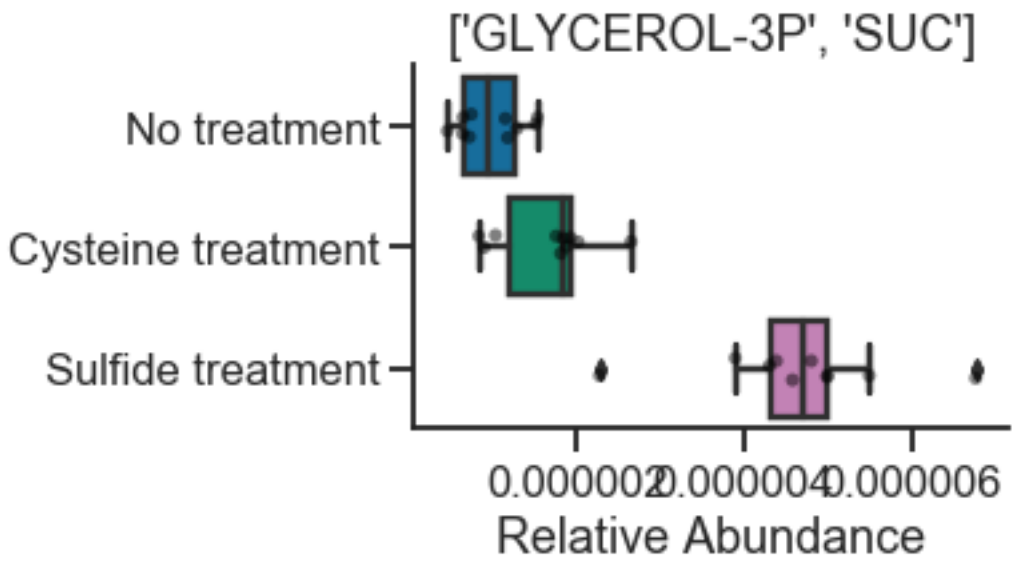

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303
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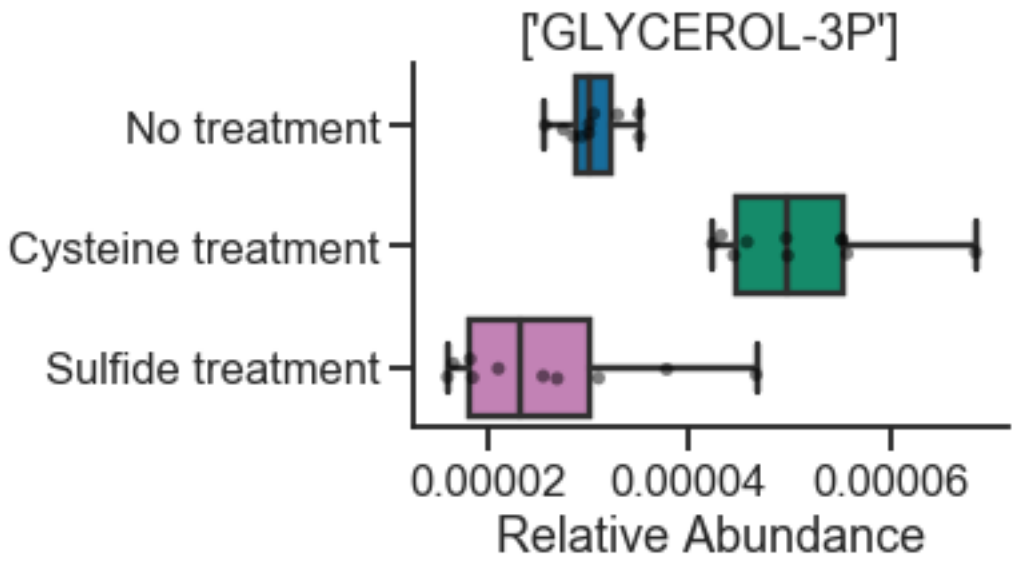


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53
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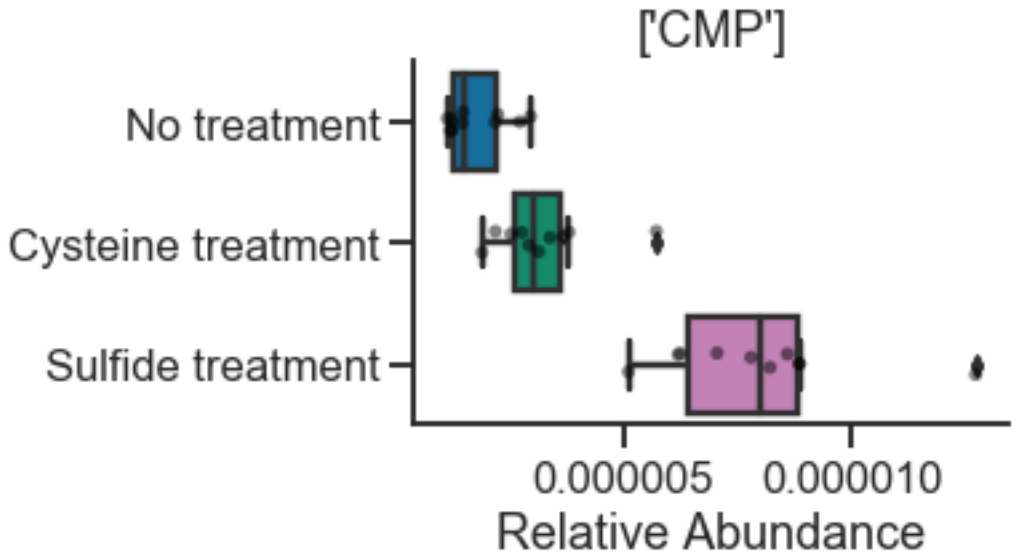

161

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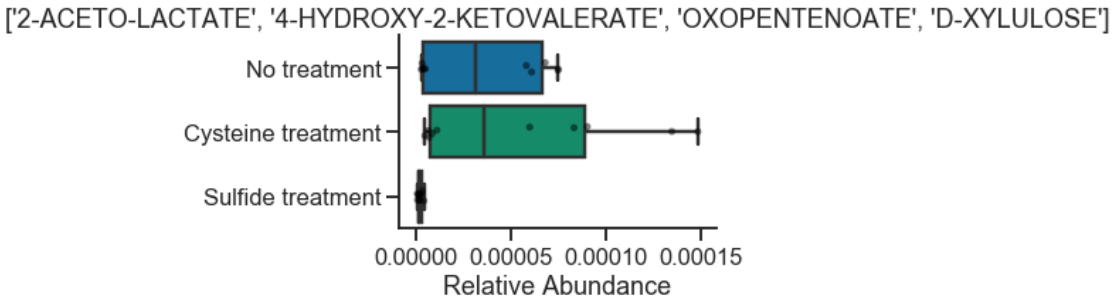

184

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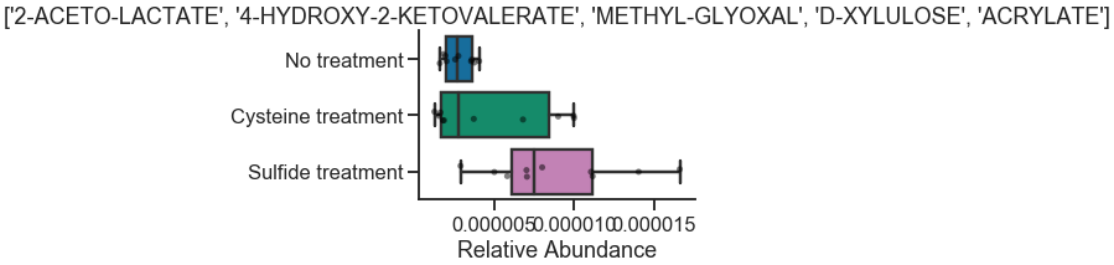

158

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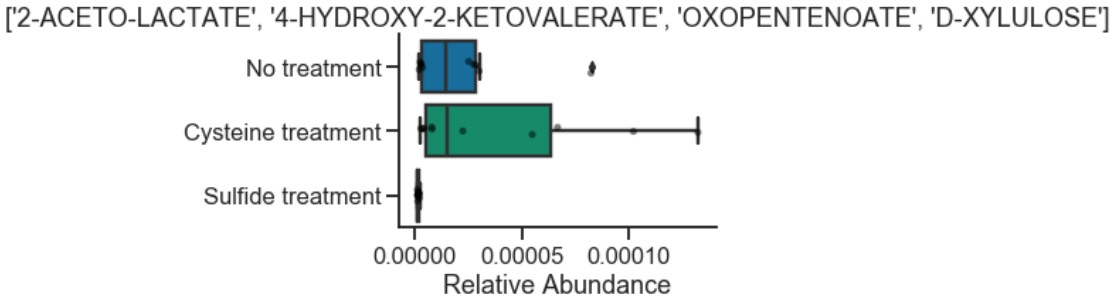

220

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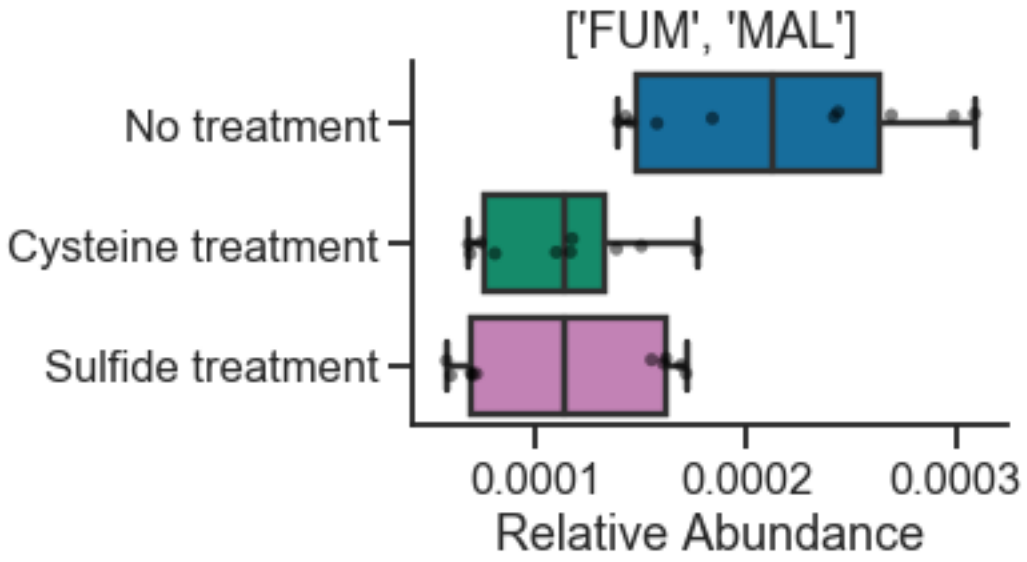

239

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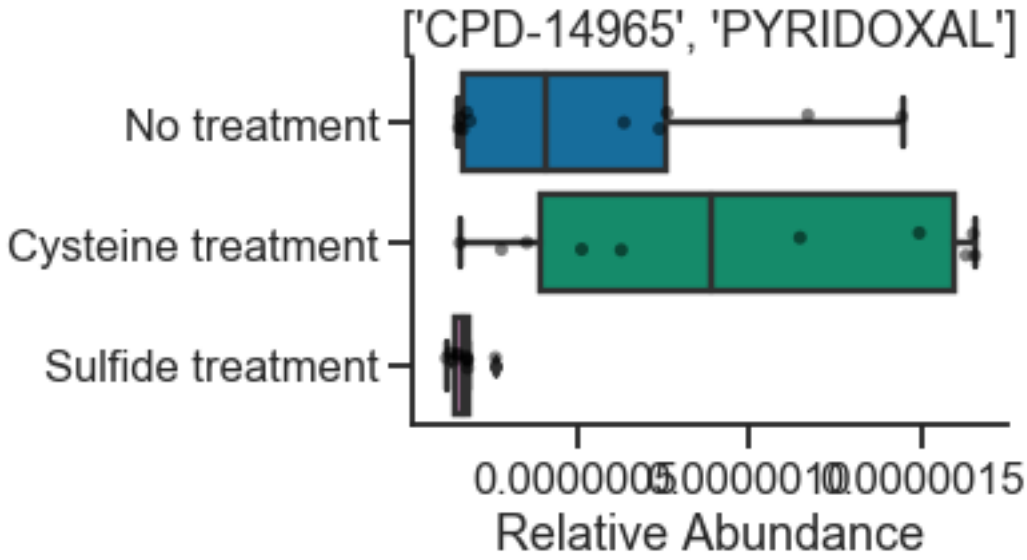

27

```
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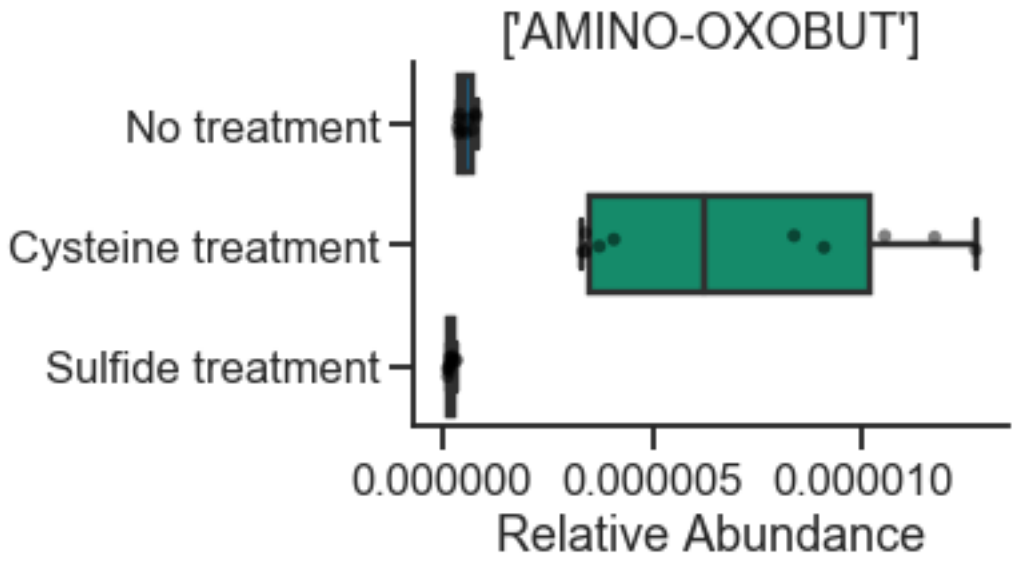


```
289
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```

[ ]: