# figures_10jan2019

April 12, 2020

## 1 mtFAS proteomics figures

### 1.0.1 Import dependencies

[1]:
```
import sys
import pandas as pd
import numpy as np

import matplotlib
import matplotlib.pyplot as plt
import seaborn as sns
sns.set(font='arial')
jakes_cmap = sns.diverging_palette(212, 61, s=99, l=77, sep=1, n=16,␣
↪center='dark') #Custom aesthetics
import xpressplot as xp
import plotly
import plotly.offline as py
import plotly_express as px
%matplotlib inline

import sklearn
from sklearn import preprocessing
import scipy
import scipy.stats as stats
```

[2]:
```
print("Pandas:",pd.__version__)
print("Numpy:",np.__version__)
print("matplotlib:",matplotlib.__version__)
print("Seaborn:",sns.__version__)
print("XPRESSplot:",xp.__version__)
print("Plotly:",plotly.__version__)
print("Scikit-learn:",sklearn.__version__)
print("scipy:",scipy.__version__)
```

```
Pandas: 1.0.2
Numpy: 1.17.4
matplotlib: 3.1.1
```

```
Seaborn: 0.10.0
XPRESSplot: 0.2.5
Plotly: 4.5.0
Scikit-learn: 0.22.1
scipy: 1.4.1
```

### 1.0.2 Import data

[3]:
```
# Read in csv (already has a genometric mean normalization applied)
df = pd.read_csv("./_data/mtfas_sum.csv",sep=",")
df = df[[
    'Gene Symbol',
    'GFP5-1 Sum',
    'GFP5-2 Sum',
    'GFP9-1 Sum',
    'GFP9-2 Sum',
    'Me2.14-1 Sum',
    'Me2.14-2 Sum',
    'Me3.8-1 Sum',
    'Me3.8-2 Sum',
    'O2.9-1 Sum',
    'O2.9-2 Sum']]
df = df.dropna(axis=0)
```

[4]:
```
df_oxsm = df[[
    'Gene Symbol',
    'GFP5-1 Sum',
    'GFP5-2 Sum',
    'GFP9-1 Sum',
    'GFP9-2 Sum',
    'O2.9-1 Sum',
    'O2.9-2 Sum']]
df_mecr = df[[
    'Gene Symbol',
    'GFP5-1 Sum',
    'GFP5-2 Sum',
    'GFP9-1 Sum',
    'GFP9-2 Sum',
    'Me2.14-1 Sum',
    'Me2.14-2 Sum',
    'Me3.8-1 Sum',
    'Me3.8-2 Sum']]

df_oxsm = df_oxsm.set_index('Gene Symbol')
df_mecr = df_mecr.set_index('Gene Symbol')
```

[5]:
```
df_oxsm.mean(axis=0)
```

[5]:
```
GFP5-1 Sum    1389.076124
GFP5-2 Sum    1389.514343
GFP9-1 Sum    1389.547701
GFP9-2 Sum    1389.103826
O2.9-1 Sum    1389.088495
O2.9-2 Sum    1389.793508
dtype: float64
```

[6]:
```
df_mecr.mean(axis=0)
```

[6]:
```
GFP5-1 Sum      1389.076124
GFP5-2 Sum      1389.514343
GFP9-1 Sum      1389.547701
GFP9-2 Sum      1389.103826
Me2.14-1 Sum    1388.877011
Me2.14-2 Sum    1389.368541
Me3.8-1 Sum     1389.171612
Me3.8-2 Sum     1389.771066
dtype: float64
```

### 1.0.3 SCALE GENES FOR HEATMAP

Used a different methodology where I split up the dataframe to GFP + MECR and GFP + OXSM and then scaled datasets
Used original sum datasheet where metrics not normalized yet
Whether or not this works is still debatable, but if not using heatmaps for publication, not an issue
Volcano plots are unchanged by this method as they are not scaled

[7]:
```
#Average every two cols together

df_mecr['GFP_b1'] = df_mecr[['GFP5-1 Sum','GFP5-2 Sum']].mean(axis=1)
df_mecr['GFP_b2'] = df_mecr[['GFP9-1 Sum','GFP9-2 Sum']].mean(axis=1)
df_mecr['MECR_b1'] = df_mecr[['Me2.14-1 Sum','Me2.14-2 Sum']].mean(axis=1)
df_mecr['MECR_b2'] = df_mecr[['Me3.8-1 Sum','Me3.8-2 Sum']].mean(axis=1)

df_oxsm['GFP_b1'] = df_oxsm[['GFP5-1 Sum','GFP5-2 Sum']].mean(axis=1)
df_oxsm['GFP_b2'] = df_oxsm[['GFP9-1 Sum','GFP9-2 Sum']].mean(axis=1)
df_oxsm['OXSM_b1'] = df_oxsm[['O2.9-1 Sum','O2.9-2 Sum']].mean(axis=1)

df_mecr_collapsed = df_mecr[['GFP_b1','GFP_b2','MECR_b1','MECR_b2']]
df_oxsm_collapsed = df_oxsm[['GFP_b1','GFP_b2','OXSM_b1']]

#Scale proteins
df_mecr_scaled = df_mecr_collapsed.copy()
```

```
df_mecr_scaled[df_mecr_scaled.columns] = preprocessing.
 ↪scale(df_mecr_scaled[df_mecr_scaled.columns],axis=1)
print("Mecr")
print(df_mecr_scaled.mean(axis=1).head())

print("\n")
df_oxsm_scaled = df_oxsm_collapsed.copy()
df_oxsm_scaled[df_oxsm_scaled.columns] = preprocessing.
 ↪scale(df_oxsm_scaled[df_oxsm_scaled.columns],axis=1)
print("Oxsm")
print(df_oxsm_scaled.mean(axis=1).head())
```

```
Mecr
Gene Symbol
Tbc1d25   -9.436896e-16
Cul4b      4.163336e-16
Dhx8      -1.026956e-15
Pgap3      9.159340e-16
Arfgef2   -8.881784e-16
dtype: float64


Oxsm
Gene Symbol
Tbc1d25    1.702342e-15
Cul4b      2.960595e-16
Dhx8       0.000000e+00
Pgap3      6.476301e-16
Arfgef2    5.366078e-15
dtype: float64
```

[8]:
```
#Generate GFP-zeroed fold changes for heatmaps

df_mecr_logFC = df_mecr.copy()
df_mecr_logFC.index.name = None
df_mecr_logFC['gfp_mean'] = df_mecr_logFC[['GFP_b1','GFP_b2',]].mean(axis=1)
df_mecr_logFC = df_mecr_logFC[['GFP_b1','GFP_b2','MECR_b1','MECR_b2']].
 ↪div(df_mecr_logFC.gfp_mean, axis=0)
df_mecr_logFC = np.log2(df_mecr_logFC)
df_mecr_logFC['base'] = df_mecr_logFC[['GFP_b1','GFP_b2']].mean(axis=1)
df_mecr_logFC = df_mecr_logFC[~df_mecr_logFC.index.duplicated()]
df_mecr_logFC = df_mecr_logFC[['GFP_b1','GFP_b2','MECR_b1','MECR_b2']].
 ↪subtract(df_mecr_logFC.base, axis=0)

df_oxsm_logFC = df_oxsm.copy()
df_oxsm_logFC.index.name = None
df_oxsm_logFC['gfp_mean'] = df_oxsm_logFC[['GFP_b1','GFP_b2',]].mean(axis=1)
```

```
df_oxsm_logFC = df_oxsm_logFC[['GFP_b1','GFP_b2','OXSM_b1']].div(df_oxsm_logFC.
 ↪gfp_mean, axis=0)
df_oxsm_logFC = np.log2(df_oxsm_logFC)
df_oxsm_logFC['base'] = df_oxsm_logFC[['GFP_b1','GFP_b2']].mean(axis=1)
df_oxsm_logFC = df_oxsm_logFC[~df_oxsm_logFC.index.duplicated()]
df_oxsm_logFC = df_oxsm_logFC[['GFP_b1','GFP_b2','OXSM_b1']].
 ↪subtract(df_oxsm_logFC.base, axis=0)
```

[9]:
```
df_all_fc = df_oxsm_logFC.copy()

df_all_fc['MECR_b1'] = df_mecr_logFC['MECR_b1']
df_all_fc['MECR_b2'] = df_mecr_logFC['MECR_b2']
df_all_fc.columns = [
    'GFP 1',
    'GFP 2',
    'Oxsm (O2-9)',
    'Mecr 1 (Me2-14)',
    'Mecr 2 (Me3-8)',
]
```

CREATE GENE LISTS

[10]:
```
# Select proteins of interest
n_module = ['Ndufv1','Ndufv2','Ndufs1','Ndufa2','Ndufs4',
            'Ndufa12','Ndufs6','Ndufv3']
q_module = ['Ndufa6','Ndufa7','Ndufa5','Ndufs2',
            'Ndufs3','Ndufs7','Ndufs8','Ndufa9']
p_module = ['Mtnd1','Ndufa3','Ndufa8','Ndufa13','Ndufa1',
            'Mtnd3','Mtnd4l','Ndufc2','Ndufa10','Ndufs5',
            'Ndufa11','Ndufb6','Ndufb5','Ndufb10','Ndufb11',
            'Mtnd4','Ndufb4','Ndufb7','Ndufb3','Ndufb8',
            'Ndufb9','Ndufb2','Ndufs5']
pp_module = ['Mtnd1','Ndufa3','Ndufa8','Ndufa13','Ndufa1',
             'Mtnd4l','Mtnd3','Ndufc2','Ndufa10','Ndufs5',
             'Ndufa11']
pp1_module = ['Mtnd1','Ndufa3','Ndufa8','Ndufa13','Ndufa1']
pp2_module = ['Mtnd4l','Mtnd3','Ndufc2','Ndufa10','Ndufs5',
              'Ndufa11']
pd_module = ['Ndufb6','Ndufb5','Ndufb10','Ndufb11','Mtnd4',
             'Ndufb4','Ndufb7','Ndufb3','Ndufb8','Ndufb9',
             'Ndufb2']
pd1_module = ['Ndufb6','Ndufb5','Ndufb10','Ndufb11','Mtnd4',
              'Ndufb4']
pd2_module = ['Ndufb7','Ndufb3','Ndufb8','Ndufb9','Ndufb2']
complex_ii = ['Sdha','Sdhb','Sdhc']
complex_iii = ['Uqcrc2','Uqcrc1','Uqcrb','Uqcrq','Cyc1','Mt-CyB',
               'Uqcr10','Uqcrh','Uqcrfs1']
```

```
complex_iv = ['Mtco1','Cox5a','Cox5b','Cox6c','Mtco2',
              'Cox7a2','Cox7c','Cox6b1','Cox7a2l','Ndufa4',
              'Cox15']
complex_v = ['Atp5a1','Atp5b','Atp5c1','Atp5e','Atp5g2',
             'Mtatp6','Mtatp8','Atp5h','Atp5i','Atp5f1',
             'Atp5d','Atp5j2','Atp5j','Atp5l','Atp5o',
             'Atpif1']
fes_cluster = ['Lyrm4','Nfs1','Iscu']
lyr_proteins = ['Ndufa6','Ndufb9','Lyrm4']
lyr_targets = ['Sdhb','Uqcrfs1']

etc = n_module + q_module + pp_module + pd_module+ complex_ii + complex_iii +␣
↪complex_iv + complex_v
```

[11]:
```
mc = pd.read_csv("./_data/Mouse_MitoCarta2_0.csv",sep=",")

volcano_mitocarta = mc['Symbol'].tolist()
```

[12]:
```
df_all_fc.head()
```

[12]:

| | GFP 1 | GFP 2 | Oxsm (O2-9) | Mecr 1 (Me2-14) | Mecr 2 (Me3-8) |
|---|---|---|---|---|---|
| Tbc1d25 | 0.054680 | -0.054680 | 0.198497 | 0.057906 | 0.214379 |
| Cul4b | -0.077994 | 0.077994 | 0.483798 | 0.291667 | 0.279313 |
| Dhx8 | -0.124763 | 0.124763 | 0.100311 | 0.357097 | 0.306092 |
| Pgap3 | -0.173520 | 0.173520 | 0.061596 | 0.136775 | -0.153875 |
| Arfgef2 | 0.049477 | -0.049477 | -0.042001 | -0.000076 | -0.137610 |

HEATMAPS

[13]:
```
# Remove gene name duplicates
df_all_fc_nodups = df_all_fc[~df_all_fc.index.duplicated()]
df_all_fc_heat = df_all_fc_nodups.reindex(labels=etc,axis=0)
df_all_fc_heat = df_all_fc_heat.dropna(axis=0)

# Get sample info
info = pd.DataFrame()
info[0] = [
    'GFP 1',
    'GFP 2',
    'Oxsm (O2-9)',
    'Mecr 1 (Me2-14)',
    'Mecr 2 (Me3-8)',
]
info[1] = [
    'GFP',
    'GFP',
    'Oxsm',
```

```
    'Mecr',
    'Mecr'
]

#Create a samples color dictionary for plots
colors = {
    'GFP':'#1b9e77',
    'Oxsm':'#d95f02',
    'Mecr':'#7570b3'}

# Generate heatmap
xp.heatmap(
    df_all_fc_heat,
    info,
    sample_palette=colors,
    figsize=(1.3,12),
    row_cluster=False,
    col_cluster=False,
    font_scale=.7,
    cbar_kws={
        'label': 'log$_2$(Fold Change)',
        'shrink': 0.2,
        'aspect': 10})

# Add the legend manually to the current Axes.
f = lambda m,c: plt.plot([],[],marker='o', color=c, ls="none")[0]
handles = [f("s", list(colors.values())[i]) for i in range(len(list(colors.
 ↪values())))]
first_legend = plt.legend(handles, list(colors.keys()), bbox_to_anchor=(15, 0.
 ↪5), loc=2, borderaxespad=0., title='Samples')
ax = plt.gca().add_artist(first_legend)

ax.figure.savefig(
    "./_figures/all_FC_heatmap.pdf",
    dpi=1800,
    bbox_inches='tight')
```

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

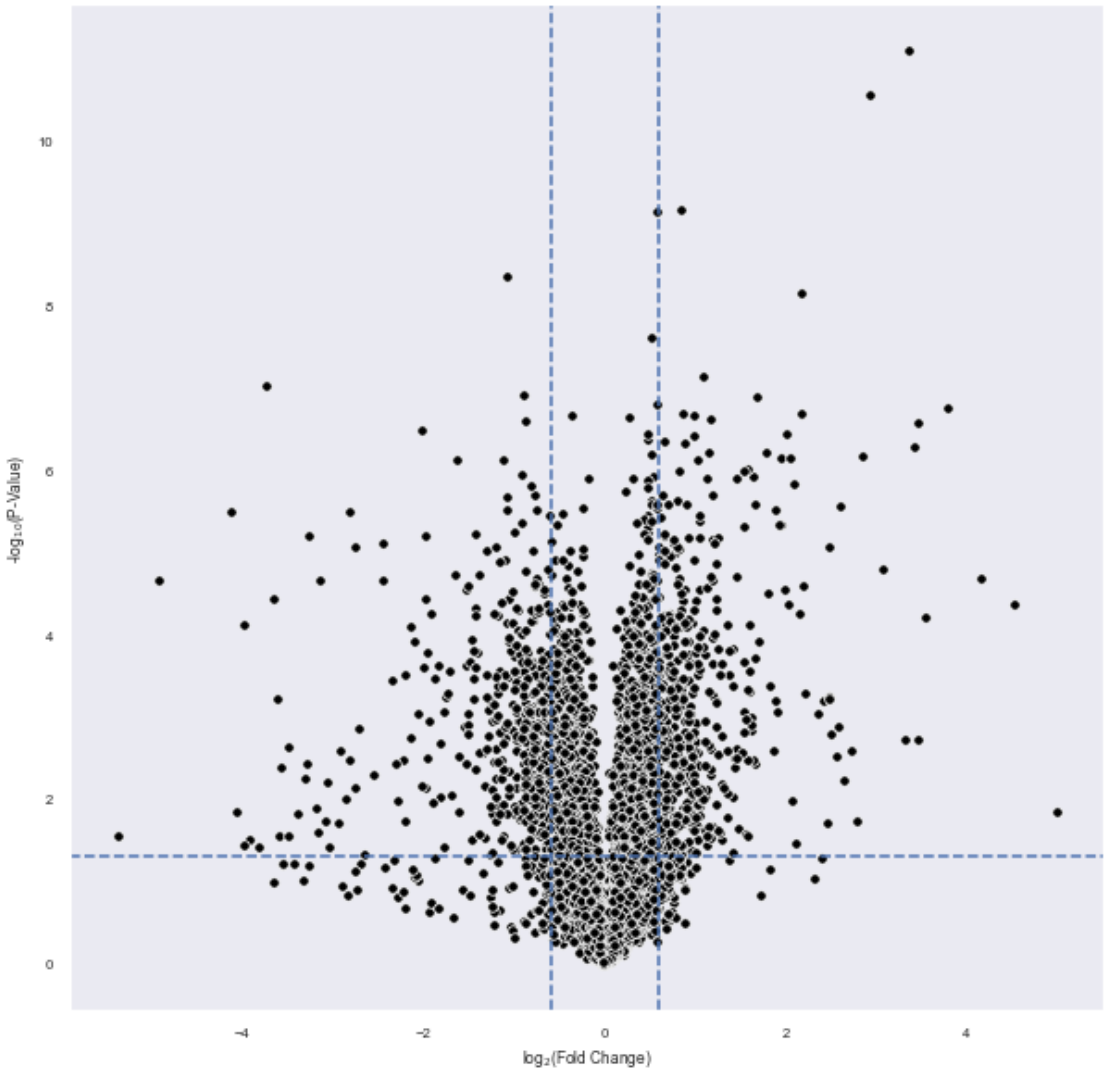
0.5
0.0
−0.5
−1.0
−1.5
−2.0
log2(Fold Change)
Samples
GFP
Oxsm
Mecr
samples
Ndufv1
Ndufv2
Ndufs1
Ndufa2
Ndufs4
Ndufa12
Ndufs6
Ndufv3
Ndufa6
Ndufa7
Ndufa5
Ndufs2
Ndufs3
Ndufs7
Ndufs5
Ndufa9
Mtnd1
Ndufa3
Ndufa8
Ndufa13
Ndufa1
Mtnd4l
Mtnd3
Ndufc2
Ndufa10
Ndufs5
Ndufa11
Ndufb6
Ndufb5
Ndufb10
Ndufb11
Mtnd4
Ndufb4
Ndufb7
Ndufb3
Ndufb8
Ndufb9
Ndufb2
Sdha
Sdhb
Sdhc
Uqcrc2
Uqcrc1
Uqcrb
Uqcrq
Cyc1
Uqcr10
Uqcrh
Uqcrfs1
Mtco1
Cox5a
Cox5b
Cox6c
Mtco2
Cox7a2
Cox7c
Cox6b1
Cox7a2l
Ndufa4
Cox15
Atp5a1
Atp5b
Atp5c1
Atp5e
Atp5g2
Mtatp6
Mtatp8
Atp5h
Atp5l
Atp5f1
Atp5d
Atp5j2
Atp5j
Atp5l
Atp5o
Atpif1
GFP 1
GFP 2
Oxsm (Q2-9)
Mecr 1 (M62-14)
Mecr 2 (Me3-5)

VOLCANO PLOTS

[14]:
```
# Get relevant data
mecr_meta = {}
for x in df_mecr.columns:
    mecr_meta[x] = x[:3]

# Remove duplicates
df_mecr_nodups = df_mecr[~df_mecr.index.duplicated()]
df_mecr_nodups = df_mecr_nodups.dropna(axis=0)

mecr_meta = pd.DataFrame.from_dict(mecr_meta, orient='index', columns=['0'])
mecr_meta = mecr_meta.reset_index()
mecr_meta.columns = [0,1]

df_mecr_nodups.index.name = None
```

[15]:
```
mecr_data = xp.volcano(
    df_mecr_nodups,
    mecr_meta,
    'MEC',
    'GFP',
    y_threshold=1.32,
    x_threshold=[-0.59,0.59],
    return_data=True)
```

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




[16]:
```
mitocarta_up = mecr_data.loc[mecr_data.index.isin(volcano_mitocarta)].
 ↪loc[(mecr_data["log$_2$(Fold Change)"] > 0.59) &␣
 ↪(mecr_data["-log$_1$$_0$(P-Value)"] > 1.31)]
print("MitoCarta up:", mitocarta_up.shape[0])

mitocarta_down = mecr_data.loc[mecr_data.index.isin(volcano_mitocarta)].
 ↪loc[(mecr_data["log$_2$(Fold Change)"] < -0.59) &␣
 ↪(mecr_data["-log$_1$$_0$(P-Value)"] > 1.31)]
print("MitoCarta down:", mitocarta_down.shape[0])
```

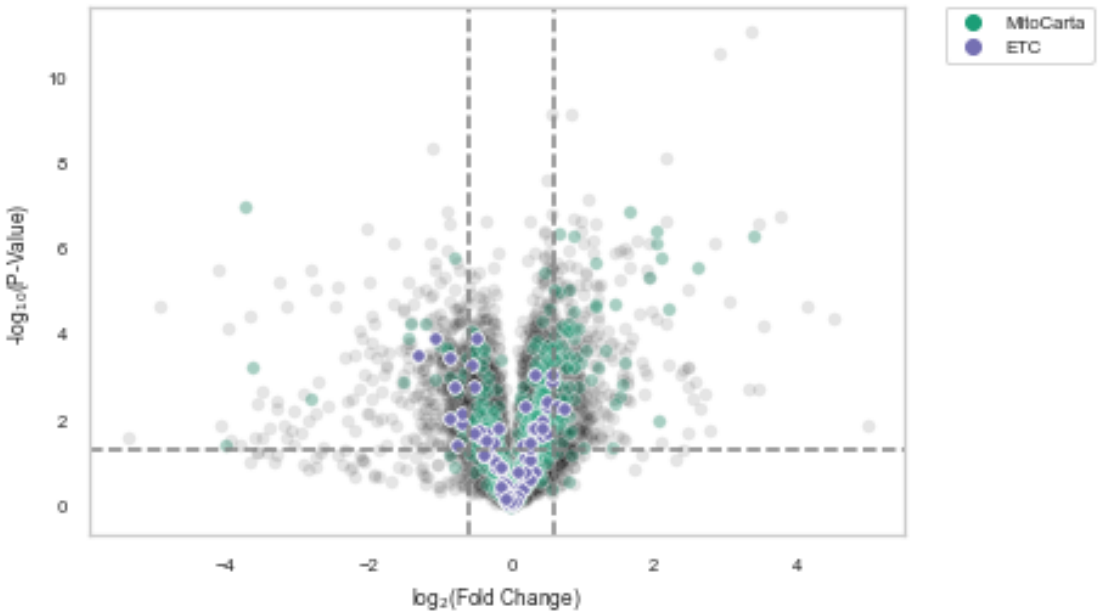
```
MitoCarta up: 77
MitoCarta down: 34
```

[17]:
```
etc_up = mecr_data.loc[mecr_data.index.isin(etc)].loc[(mecr_data["log$_2$(Fold␣
 ↪Change)"] > 0.59) & (mecr_data["-log$_1$$_0$(P-Value)"] > 1.31)]
print("ETC up:", etc_up.shape[0])

etc_down = mecr_data.loc[mecr_data.index.isin(etc)].
 ↪loc[(mecr_data["log$_2$(Fold Change)"] < -0.59) &␣
 ↪(mecr_data["-log$_1$$_0$(P-Value)"] > 1.31)]
print("ETC down:", etc_down.shape[0])
```

```
ETC up: 2
ETC down: 8
```

[18]:
```
xp.volcano(
    df_mecr_nodups,
    mecr_meta,
    'MEC',
    'GFP',
    highlight_points=[volcano_mitocarta,etc],
    highlight_color=[
        '#1b9e77',
        '#7570b3'],
    highlight_names=[
        'MitoCarta',
        'ETC'],
    alpha=.1,
    alpha_highlights=[0.3,1],
    y_threshold=1.31,
    x_threshold=[-0.59,0.59],
    threshold_color='grey',
    dpi=1800,
    figsize=(6,4),
    save_fig='./_figures/mecr_volcano.pdf',
    whitegrid=True)
```

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




[19]:
```
volcano_stem = ['Cdh15', 'Cd34', 'MyoD', 'Tnnt3', 'Tnni2',
                    'CD44', 'Tnnt2', 'Tnni1', 'Tnnc1', 'Myh1',
                    'Mb', 'Chdh', 'Mylpf', 'Myh3', 'Acta1',
                    'Myl1', 'Myl4', 'Myl6b', 'Sorbs2', 'Csrp3',
                    'Nrap', 'Klh141', 'Ccdc141', 'Neb', 'Bin1',
                    'Ckm', 'Ckb', 'Ank1', 'Trim72', 'Itga7',
                    'Akap6', 'Dusp27']
```

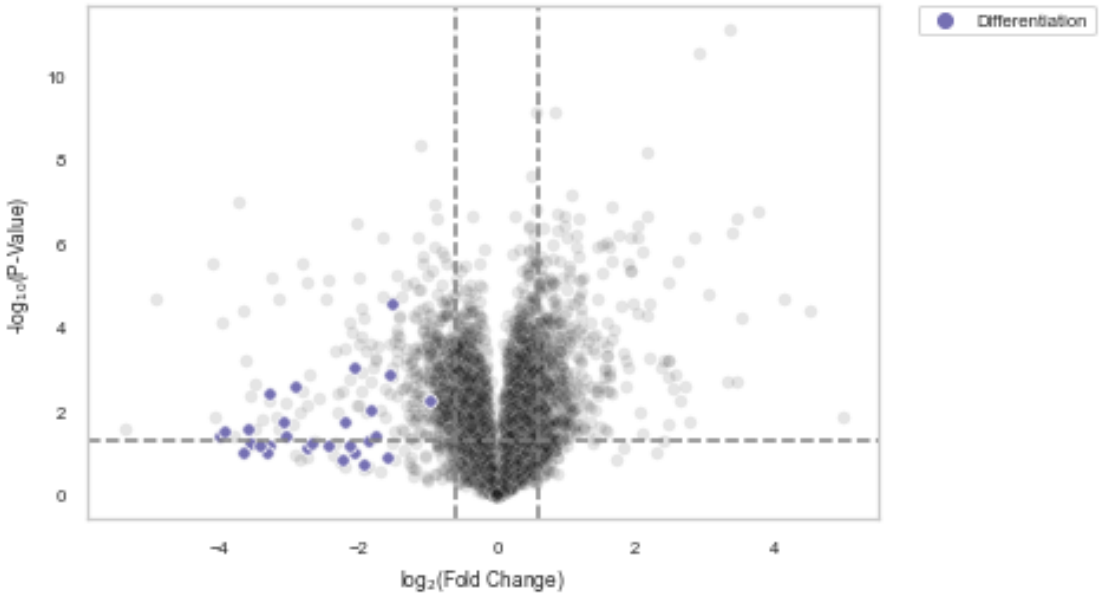
[20]:
```
stem_up = mecr_data.loc[mecr_data.index.isin(volcano_stem)].
 ↪loc[(mecr_data["log$_2$(Fold Change)"] > 0.59) &␣
 ↪(mecr_data["-log$_1$$_0$(P-Value)"] > 1.31)]
print("Differentiation up:", stem_up.shape[0])

stem_down = mecr_data.loc[mecr_data.index.isin(volcano_stem)].
 ↪loc[(mecr_data["log$_2$(Fold Change)"] < -0.59) &␣
 ↪(mecr_data["-log$_1$$_0$(P-Value)"] > 1.31)]
print("Differentiation down:", stem_down.shape[0])
```

```
Differentiation up: 0
Differentiation down: 14
```

[21]:
```
xp.volcano(
    df_mecr_nodups,
    mecr_meta,
    'MEC',
    'GFP',
```

```
    highlight_points=[volcano_stem],
    highlight_color=[
        '#7570b3'],
    highlight_names=[
        'Differentiation'],
    alpha=.1,
    alpha_highlights=[1],
    y_threshold=1.31,
    x_threshold=[-0.59,0.59],
    threshold_color='grey',
    dpi=1800,
    figsize=(6,4),
    save_fig='./_figures/mecr_volcano_differentiation.pdf',
    whitegrid=True)
```

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

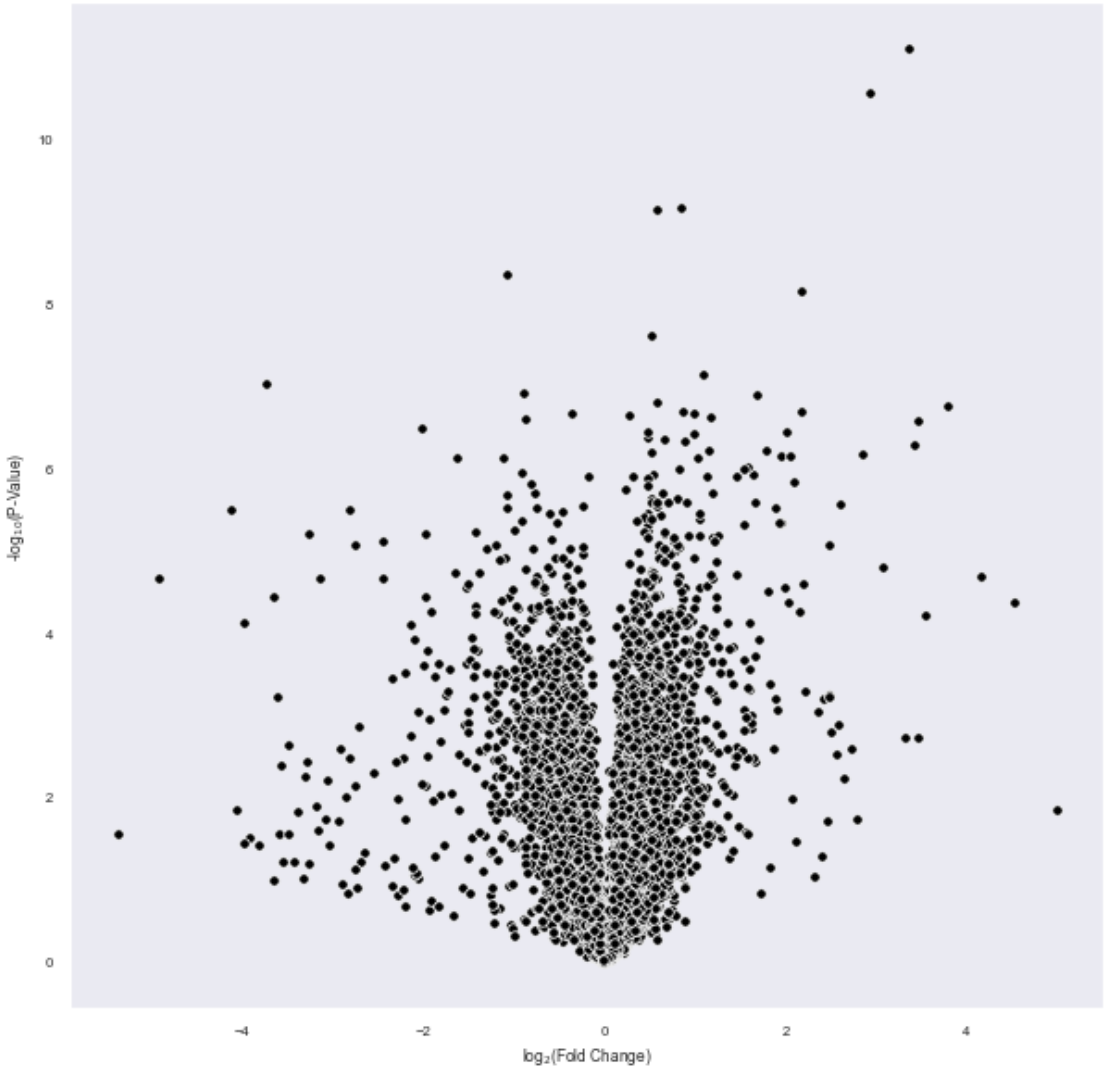



### Generate interactive Mecr volcano plot

[22]:
```
# Get X and Y data
data = xp.volcano(
    df_mecr_nodups,
    mecr_meta,
    'MEC',
    'GFP',
    return_data=True)
```

```
# Prep labels
data['names'] = data.index.tolist()
label_list = []

for index, row in data.iterrows():

    if row[2] in etc:
        label_list.append('ETC')
    elif row[2] in volcano_mitocarta:
        label_list.append('Mitocarta')
    else:
        label_list.append('Other')

data['label'] = label_list

# Plot
sc = px.scatter(
    data,
    x='log$_2$(Fold Change)',
    y='-log$_1$$_0$(P-Value)',
    hover_name='names',
    color="label",
    color_discrete_map={
        'ETC': "#8b0000",
        'Mitocarta': "#6666ff",
        'Other': "#D3D3D3"
    },
    labels={
        'log$_2$(Fold Change)': 'log<sub>2</sub>(Fold Change)',
        '-log$_1$$_0$(P-Value)': '-log<sub>10</sub>(P-Value)'
    },
    log_x=False,
    log_y=False,
    opacity=0.7,
    width=1400,
    height=1000,
    title="Mecr proteomics")

py.offline.plot(sc, filename='./_figures/mecr_interactive.html')
```

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




[22]: `'./_figures/mecr_interactive.html'`

[ ]: