main

March 17, 2023

1 Calculate RXE for public dataset, females only based on Apua's script

This has been edited from Apua's script.

[1]:

```
import numpy as np
import pandas as pd
from os import environ
from plotnine import *
from pyhere import here
import re, session_info
import statsmodels.api as sm
from functools import lru_cache
from scipy.stats import variation
from warnings import filterwarnings
from scipy.stats import mannwhitneyu
from statsmodels.formula.api import ols
from matplotlib.cbook import mplDeprecation
```

[2]:

```
filterwarnings('ignore',category=mplDeprecation)
filterwarnings('ignore', category=UserWarning, module='plotnine.*')
filterwarnings('ignore', category=DeprecationWarning, module='plotnine.*')
```

[3]:

```
environ['NUMEXPR_MAX_THREADS'] = '4'
```

1.1 Functions

[4]:

```
@lru_cache()
def get_pheno():
    return pd.read_csv(here('input/phenotypes/_m/phenotypes.csv'), index_col=0)


@lru_cache()
def get_logTPM(tissue):
    fn = here(f'input/counts/text_files_counts/tpm/_m/{tissue}/gene.log2tpm.
 ↪csv')
```

```
    return pd.read_csv(fn, index_col=0)


@lru_cache()
def get_annotation():
    """
    Get the annotation file for genes.
    """
    fn = here('input/counts/text_files_counts/_m',
              'caudate/gene_annotation.txt')
    return pd.read_csv(fn, sep='\t')
```

[5]:

```
def save_ggplot(p, fn):
    for ext in ['.pdf', '.png', '.svg']:
        p.save(fn+ext, width=10, height=7)


def rge(df, chrom):
    return df[df['seqname']==chrom][samples].mean() \
    - df[(df['chrom_type']=='autosome') & (df['seqname']!=chrom)][samples].
 ↪mean()


def annotate_chrom(df):
    df.loc[:, 'chrom_types'] = 'Other'
    df.loc[df['seqnames'].isin(['chrX', 'chrY']), 'chrom_types'] = 'Allosome'
    df.loc[df['seqnames'].str.contains('chr\d+'), 'chrom_types'] = 'Autosome'
    df.loc[df['seqnames'] == 'chrM', 'chrom_types'] = 'Mitochondria'
    df.loc[df['seqnames'] == 'chrX', 'chrom_types'] = 'X'
    return df
```

1.2 Load and merge data

[6]:

```
log2tpm = pd.DataFrame()
for tissue in ['caudate', 'dlpfc', 'hippocampus']:
    log2tpm = pd.concat([log2tpm, get_logTPM(tissue)], axis=1)
print(log2tpm.shape)
log2tpm.iloc[0:2, 0:5]
```

```
(52569, 1217)
```

[6]:

```
                            R12864    R12865    R12866    R12867    R12868
name
TSPAN6|ENSG00000000003.15  2.441001  3.844446  3.045605  2.788269  2.901279
TNMD|ENSG00000000005.6     0.000000  0.000000  0.082527  0.000000  0.000000
```

1.3 Annotate TPM

[7]:

```
get_annotation().head(2)
```

[7]:

```
                        name seqnames      start        end  width gene_name
0  TSPAN6|ENSG00000000003.15     chrX  100627108  100639991  12884    TSPAN6
1     TNMD|ENSG00000000005.6     chrX  100584936  100599885  14950      TNMD
```

[8]:

```
df0 = log2tpm.merge(get_annotation().loc[:, ['name', 'gene_name', 'seqnames']],
                    left_index=True, right_on='name')
df0 = annotate_chrom(df0)
print(df0.shape)
df0.groupby('chrom_types').size()
```

```
(52569, 1221)
```

[8]:

```
chrom_types
Allosome          501
Autosome        50034
Mitochondria       14
X                2020
dtype: int64
```

1.4 Filtering genes

[9]:

```
samples = [x for x in log2tpm.columns if re.match('R\d+', x)]
df = df0[(df0[samples].sum(axis=1) >= 0.2 * len(samples)) &
         (df0['chrom_types'].isin(['X', 'Autosome']))].copy()
df.shape
```

[9]:

```
(24499, 1221)
```

1.5 Generate RXE

[10]:

```
df1  = df.groupby('chrom_types').mean(numeric_only=True).transpose()
df1.head(2)
```

[10]:

```
chrom_types  Autosome         X
R12864       2.332196  2.461731
R12865       2.740215  2.829693
```

[11]:

```
df1['sample'] = df1.index
df1['RXE'] = df1['X'] - df1['Autosome']
df1.head(2)
```

```
[11]: chrom_types  Autosome         X  sample       RXE
      R12864       2.332196  2.461731  R12864  0.129535
      R12865       2.740215  2.829693  R12865  0.089478
```

1.6 Annotated sample IDs

```
[12]: df2 = df1.merge(get_pheno().loc[:, ['RNum', 'Sex', 'Dx', 'Region']],
                      left_index=True, right_on='RNum')
      df2.loc[:, ['Region', 'Sex', 'RXE']].groupby(['Region', 'Sex']).describe()
```

```
[12]:                RXE                                                    \
                   count      mean       std       min       25%       50%
      Region  Sex
      Caudate F    129.0  0.072434  0.066423 -0.116318  0.037158  0.087259
              M    289.0  0.073250  0.055947 -0.144156  0.046930  0.085875
      DLPFC   F    125.0  0.105212  0.042601 -0.026092  0.089677  0.119341
              M    263.0  0.102371  0.068521 -0.919013  0.093479  0.109438
      HIPPO   F    132.0  0.111335  0.032613  0.018946  0.094986  0.114084
              M    276.0  0.106895  0.032326 -0.005364  0.090518  0.112290


                        75%       max
      Region  Sex
      Caudate F    0.120710  0.180794
              M    0.111217  0.162486
      DLPFC   F    0.132669  0.171622
              M    0.124097  0.155076
      HIPPO   F    0.133184  0.178053
              M    0.127329  0.208088
```

1.7 Metrics summary

1.7.1 Variation of RXE

```
[13]: for tissue in ['Caudate', 'DLPFC', 'HIPPO']:
          for sex in ['F', 'M']:
              var = variation(df2[(df2['Region']==tissue) & (df2['Sex']==sex)].RXE)
              print(f'There is {var:.3} variation for {sex} in {tissue}.')
```

```
There is 0.913 variation for F in Caudate.
There is 0.762 variation for M in Caudate.
There is 0.403 variation for F in DLPFC.
There is 0.668 variation for M in DLPFC.
There is 0.292 variation for F in HIPPO.
There is 0.302 variation for M in HIPPO.
```

[14]:

```
## Separate out tissues

caudate = df2[(df2['Region']=='Caudate')].copy()
dlpfc = df2[(df2['Region']=='DLPFC')].copy()
hippo = df2[(df2['Region']=='HIPPO')].copy()
```

1.7.2 Mann-WhitneyU (Female to Male)

[15]:

```
for tissue in ['caudate', 'DLPFC', 'hippocampus']:
    df_config = {'caudate': caudate,'DLPFC': dlpfc,'hippocampus': hippo}
    stat, pval =␣
  ↪mannwhitneyu(df_config[tissue][(df_config[tissue]['Sex']=='F')].RXE,
                                         ␣
  ↪df_config[tissue][(df_config[tissue]['Sex']=='M')].RXE)
    print(f'Mann-WhitneyU for female vs male (RXE) for {tissue}: {pval:.4}')
```

```
Mann-WhitneyU for female vs male (RXE) for caudate: 0.5742
Mann-WhitneyU for female vs male (RXE) for DLPFC: 0.04684
Mann-WhitneyU for female vs male (RXE) for hippocampus: 0.1329
```

1.7.3 Mann-WhitneyU (CTL vs SZ)

[16]:

```
for tissue in ['caudate', 'DLPFC', 'hippocampus']:
    df_config = {'caudate': caudate,'DLPFC': dlpfc,'hippocampus': hippo}
    stat, pval =␣
  ↪mannwhitneyu(df_config[tissue][(df_config[tissue]['Dx']=='Control')].RXE,
                                         ␣
  ↪df_config[tissue][(df_config[tissue]['Dx']=='SCZD')].RXE)
    print(f'Mann-WhitneyU for ctl vs sz (RXE) for {tissue}: {pval:.4}')
```

```
Mann-WhitneyU for ctl vs sz (RXE) for caudate: 0.6328
Mann-WhitneyU for ctl vs sz (RXE) for DLPFC: 0.0736
Mann-WhitneyU for ctl vs sz (RXE) for hippocampus: 0.004401
```

1.7.4 Subset by sex: diagnosis status

[17]:

```
for tissue in ['caudate', 'DLPFC', 'hippocampus']:
    df_config = {'caudate': caudate,'DLPFC': dlpfc,'hippocampus': hippo}
    female = df_config[tissue][(df_config[tissue]['Sex']=='F')].copy()
    male = df_config[tissue][(df_config[tissue]['Sex']=='M')].copy()
    stat_f, pval_f = mannwhitneyu(female[(female['Dx']=='Control')].RXE,
                                  female[(female['Dx']=='SCZD')].RXE)
    stat_m, pval_m = mannwhitneyu(male[(male['Dx']=='Control')].RXE,
                                  male[(male['Dx']=='SCZD')].RXE)
```

```python
    print(f'Mann-WhitneyU of female, ctl vs sz (RXE) for {tissue}: {pval_f:.4}')
    print(f'Mann-WhitneyU of male, ctl vs sz (RXE) for {tissue}: {pval_m:.4}')
```

```
Mann-WhitneyU of female, ctl vs sz (RXE) for caudate: 0.2795
Mann-WhitneyU of male, ctl vs sz (RXE) for caudate: 0.1496
Mann-WhitneyU of female, ctl vs sz (RXE) for DLPFC: 0.6639
Mann-WhitneyU of male, ctl vs sz (RXE) for DLPFC: 0.06367
Mann-WhitneyU of female, ctl vs sz (RXE) for hippocampus: 0.2548
Mann-WhitneyU of male, ctl vs sz (RXE) for hippocampus: 0.004064
```

1.7.5 X chromosome expression differences

[18]:

```python
for tissue in ['caudate', 'DLPFC', 'hippocampus']:
    df_config = {'caudate': caudate,'DLPFC': dlpfc,'hippocampus': hippo}
    female = df_config[tissue][(df_config[tissue]['Sex']=='F')].copy()
    male = df_config[tissue][(df_config[tissue]['Sex']=='M')].copy()
    stat_f, pval_f = mannwhitneyu(female[(female['Dx']=='Control')].X,
                                  female[(female['Dx']=='SCZD')].X)
    stat_m, pval_m = mannwhitneyu(male[(male['Dx']=='Control')].X,
                                  male[(male['Dx']=='SCZD')].X)
    print(f'Mann-WhitneyU of female, ctl vs sz (RXE) for {tissue}: {pval_f:.4}')
    print(f'Mann-WhitneyU of male, ctl vs sz (RXE) for {tissue}: {pval_m:.4}')
```

```
Mann-WhitneyU of female, ctl vs sz (RXE) for caudate: 0.132
Mann-WhitneyU of male, ctl vs sz (RXE) for caudate: 0.2937
Mann-WhitneyU of female, ctl vs sz (RXE) for DLPFC: 0.2231
Mann-WhitneyU of male, ctl vs sz (RXE) for DLPFC: 0.01622
Mann-WhitneyU of female, ctl vs sz (RXE) for hippocampus: 0.9944
Mann-WhitneyU of male, ctl vs sz (RXE) for hippocampus: 0.1167
```

1.7.6 Autosome expression

[19]:

```python
for tissue in ['caudate', 'DLPFC', 'hippocampus']:
    df_config = {'caudate': caudate,
                 'DLPFC': dlpfc,
                 'hippocampus': hippo}
    female = df_config[tissue][(df_config[tissue]['Sex']=='F')].copy()
    male = df_config[tissue][(df_config[tissue]['Sex']=='M')].copy()
    stat_f, pval_f = mannwhitneyu(female[(female['Dx']=='Control')].Autosome,
                                  female[(female['Dx']=='SCZD')].Autosome)
    stat_m, pval_m = mannwhitneyu(male[(male['Dx']=='Control')].Autosome,
                                  male[(male['Dx']=='SCZD')].Autosome)
    print(f'Mann-WhitneyU of female, ctl vs sz (RXE) for {tissue}: {pval_f:.4}')
    print(f'Mann-WhitneyU of male, ctl vs sz (RXE) for {tissue}: {pval_m:.4}')
```

```
Mann-WhitneyU of female, ctl vs sz (RXE) for caudate: 0.2489
Mann-WhitneyU of male, ctl vs sz (RXE) for caudate: 0.2242
Mann-WhitneyU of female, ctl vs sz (RXE) for DLPFC: 0.251
Mann-WhitneyU of male, ctl vs sz (RXE) for DLPFC: 0.006911
Mann-WhitneyU of female, ctl vs sz (RXE) for hippocampus: 0.7817
Mann-WhitneyU of male, ctl vs sz (RXE) for hippocampus: 0.6085
```

1.7.7 Interaction model

[20]:

```
df2.Sex = df2.Sex.astype('category').cat.rename_categories({'F': 'Female', 'M':␣
 ↪'Male'})
df2.Dx = df2.Dx.astype('category').cat.rename_categories({'SCZD': 'SZ',␣
 ↪'Control': 'CTL'})
df2.head(2)
```

[20]:

```
                  Autosome         X  sample       RXE    RNum     Sex  Dx  \
SAMPLE_ID
R12864_H5FM2BBXX  2.332196  2.461731  R12864  0.129535  R12864  Female  SZ
R12865_H5FM2BBXX  2.740215  2.829693  R12865  0.089478  R12865    Male  SZ

                   Region
SAMPLE_ID
R12864_H5FM2BBXX  Caudate
R12865_H5FM2BBXX  Caudate
```

[21]:

```
df3 = df2.reset_index()[['RNum', 'RXE', 'Sex', 'Dx', 'Region']].
 ↪set_index('RNum')
df3[df3.columns[1]] = df3.Sex.cat.codes
df3[df3.columns[2]] = df3.Dx.cat.codes
df3[df3.columns[3]] = df3.Region.astype('category').cat.codes
df3.head(2)
```

[21]:

```
             RXE  Sex  Dx  Region
RNum
R12864  0.129535    0   1       0
R12865  0.089478    1   1       0
```

[22]:

```
anova_df = df2.loc[:,['RXE','Sex','Dx','Region']]

# ANOVA results with combinations of 2 groups:
formula = 'RXE ~ C(Sex) + C(Dx) + C(Region) + C(Sex):C(Dx) + C(Sex):C(Region) +␣
 ↪C(Dx):C(Region)'
lm = ols(formula, anova_df).fit()
table = sm.stats.anova_lm(lm, typ=2)
print(table)
```

```
                      sum_sq      df          F        PR(>F)
C(Sex)              0.001895     1.0   0.690473  4.061684e-01
C(Dx)               0.030385     1.0  11.072889  9.024554e-04
C(Region)           0.296575     2.0  54.038442  3.354717e-23
C(Sex):C(Dx)        0.010747     1.0   3.916503  4.804177e-02
C(Sex):C(Region)    0.001777     2.0   0.323846  7.234250e-01
C(Dx):C(Region)     0.003186     2.0   0.580494  5.597787e-01
Residual            3.303914  1204.0        NaN           NaN
```

By tissue interaction

[23]:

```python
from statsmodels.stats.multicomp import pairwise_tukeyhsd
# Assumes parametric

for tissue in ['caudate', 'DLPFC', 'hippocampus']:
    df_config = {'caudate': caudate,'DLPFC': dlpfc,'hippocampus': hippo}
    anova_df = df_config[tissue].loc[:, ['RXE', 'Sex', 'Dx']]
    # ANOVA results with combinations of 2 groups:
    formula = 'RXE ~ C(Sex) + C(Dx) + C(Sex):C(Dx)'
    lm = ols(formula, anova_df).fit()
    table = sm.stats.anova_lm(lm, typ=2)
    print(tissue)
    print(table)
    print('\n')
    anova_df['combination'] = anova_df.Sex.astype('str') + ' / ' + anova_df.Dx.
 ↪astype('str')
    anova_df['combination'] = anova_df.Sex.astype('str') + ' / ' + anova_df.Dx.
 ↪astype('str')
    # perform multiple pairwise comparison (Tukey HSD)
    m_comp = pairwise_tukeyhsd(endog=anova_df['RXE'],␣
 ↪groups=anova_df['combination'], alpha=0.05)
    # coerce the tukeyhsd table to a DataFrame
    tukey_data = pd.DataFrame(data=m_comp._results_table.data[1:], columns =␣
 ↪m_comp._results_table.data[0])
    if tukey_data[(tukey_data['p-adj'] < 0.05)].shape[0] == 0:
        print('There is no significant interactions!')
    else:
        print(tukey_data[(tukey_data['p-adj'] < 0.05)])
    print('\n')
```

```
caudate
                 sum_sq     df         F    PR(>F)
C(Sex)         0.000021    1.0  0.006019  0.938196
C(Dx)          0.003460    1.0  0.983331  0.321957
C(Sex):C(Dx)   0.006188    1.0  1.758856  0.185499
Residual       1.456543  414.0       NaN       NaN
```

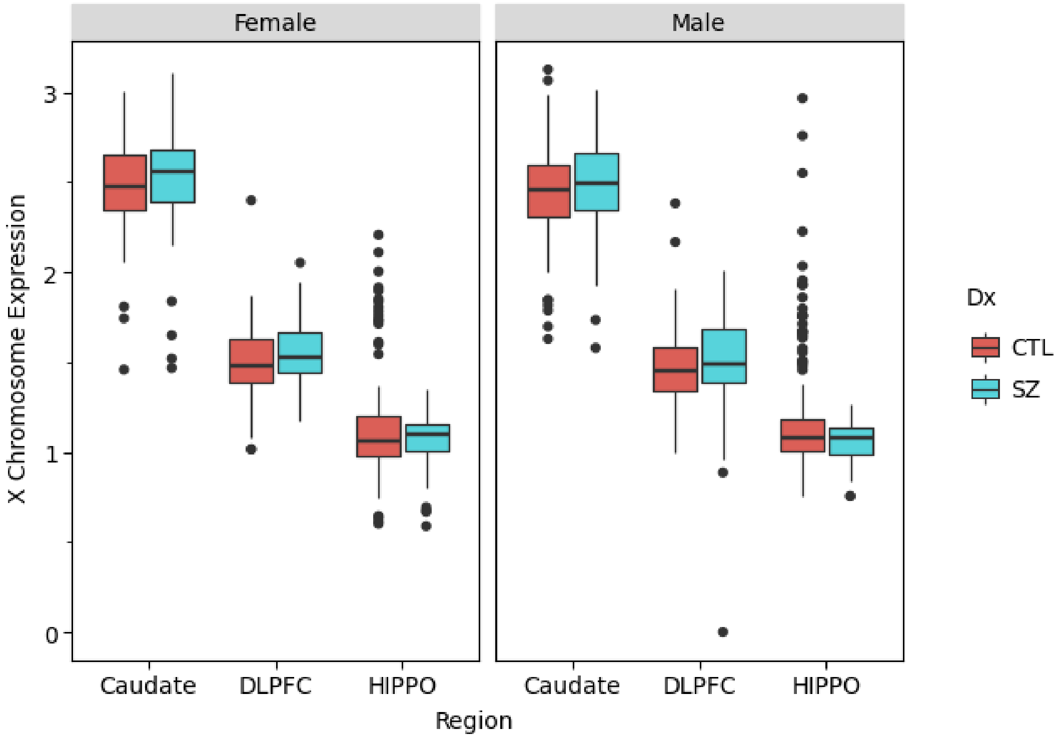
```
There is no significant interactions!


DLPFC
                 sum_sq     df         F    PR(>F)
C(Sex)         0.000870    1.0  0.232827  0.629711
C(Dx)          0.015831    1.0  4.236993  0.040226
C(Sex):C(Dx)   0.004534    1.0  1.213578  0.271315
Residual       1.434789  384.0       NaN       NaN


There is no significant interactions!


hippocampus
                 sum_sq     df          F    PR(>F)
C(Sex)         0.002564    1.0   2.516841  0.113418
C(Dx)          0.014101    1.0  13.843822  0.000227
C(Sex):C(Dx)   0.001110    1.0   1.089551  0.297195
Residual       0.411496  404.0        NaN       NaN


        group1    group2  meandiff   p-adj   lower   upper  reject
2  F / Control  M / SCZD   -0.0176  0.0022 -0.0303 -0.0049    True
5  M / Control  M / SCZD   -0.0149  0.0018 -0.0256 -0.0043    True
```

1.8 Plot RXE

[24]:

```
p = ggplot(df2, aes(x='Region', y='X', fill='Dx')) \
    + geom_boxplot() \
    + facet_grid('~Sex')\
    + ylab('X Chromosome Expression')\
    + theme_matplotlib()
p
```

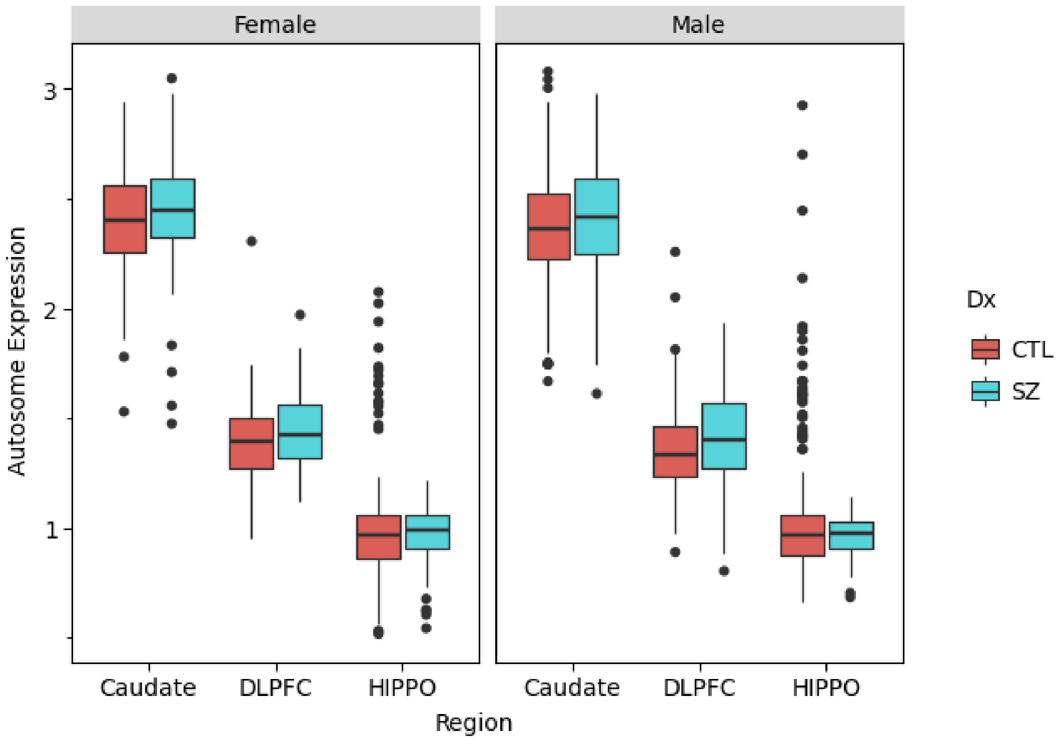
```
/users/jbenjami/.local/lib/python3.9/site-packages/plotnine/utils.py:371:
FutureWarning: The frame.append method is deprecated and will be removed from
pandas in a future version. Use pandas.concat instead.
```



```
[24]: <ggplot: (8792500059356)>
```

```
[25]: p = ggplot(df2, aes(x='Region', y='Autosome', fill='Dx')) \
          + geom_boxplot() \
          + facet_grid('~Sex')\
          + ylab('Autosome Expression')\
          + theme_matplotlib()
      p
```

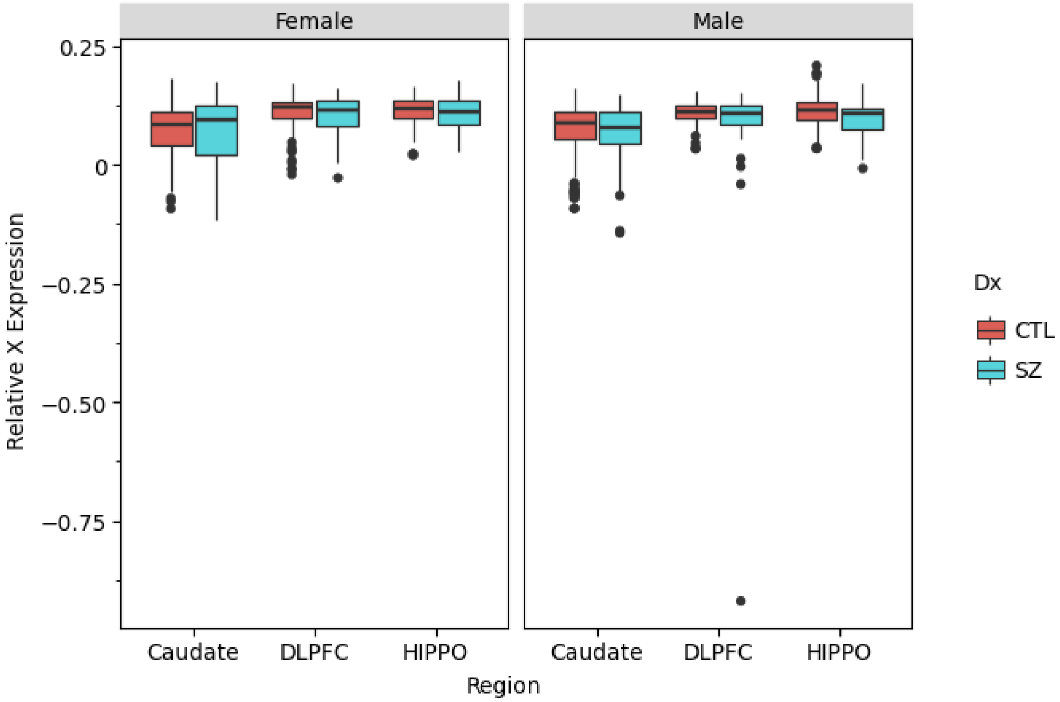
```
/users/jbenjami/.local/lib/python3.9/site-packages/plotnine/utils.py:371:
FutureWarning: The frame.append method is deprecated and will be removed from
pandas in a future version. Use pandas.concat instead.
```



```
[25]: <ggplot: (8792499901486)>
```

```
[26]: p = ggplot(df2, aes(x='Region', y='RXE', fill='Dx')) \
          + geom_boxplot() \
          + facet_grid('~Sex')\
          + ylab('Relative X Expression')\
          + theme_matplotlib()
      p
```

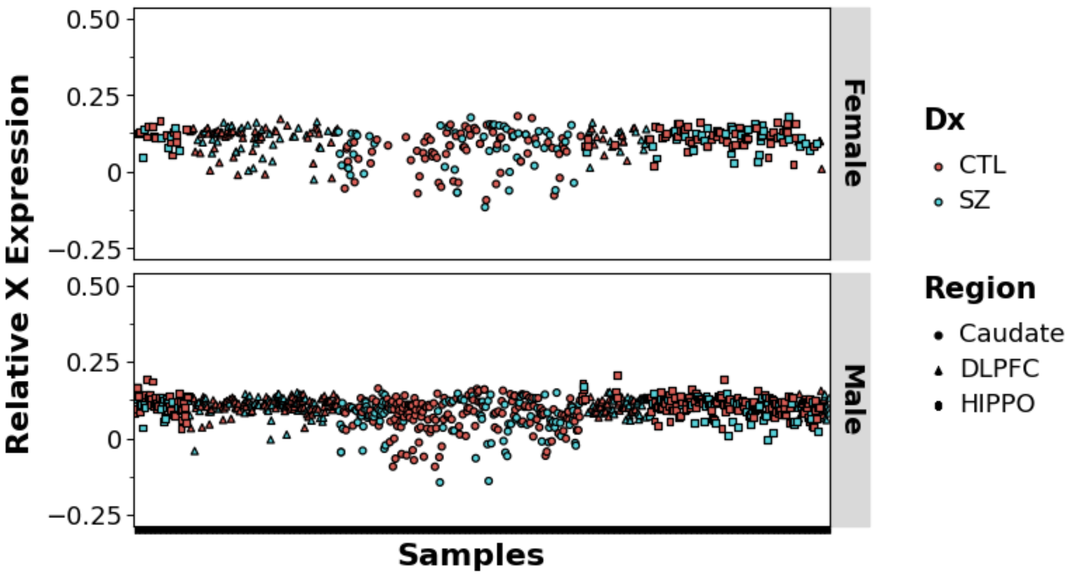
```
/users/jbenjami/.local/lib/python3.9/site-packages/plotnine/utils.py:371:
FutureWarning: The frame.append method is deprecated and will be removed from
pandas in a future version. Use pandas.concat instead.
```



```
[26]: <ggplot: (8792499801338)>
```

```
[27]: p = ggplot(df2, aes(x='sample', y='RXE', fill='Dx', shape='Region')) \
      + geom_point() + ylim([-0.25, 0.5]) + xlab('Samples')\
      + ylab('Relative X Expression') + facet_grid('Sex~.')\
      + theme_matplotlib() \
      + theme(axis_text_x=element_blank(),
              axis_text=element_text(size=13),
              legend_title=element_text(size=15, face='bold'),
              legend_text=element_text(size=13),
              axis_title=element_text(size=16, face='bold'),
              strip_text=element_text(size=14, face='bold'))
      p
```

```
/users/jbenjami/.local/lib/python3.9/site-packages/plotnine/utils.py:371:
FutureWarning: The frame.append method is deprecated and will be removed from
pandas in a future version. Use pandas.concat instead.
```



[27]: <ggplot: (8792499708100)>

```
[28]: df2.to_csv('RXE_public.csv')
      save_ggplot(p, 'RXE_public_bySex')
```

```
/users/jbenjami/.local/lib/python3.9/site-packages/plotnine/utils.py:371:
FutureWarning: The frame.append method is deprecated and will be removed from
pandas in a future version. Use pandas.concat instead.
/users/jbenjami/.local/lib/python3.9/site-packages/plotnine/utils.py:371:
FutureWarning: The frame.append method is deprecated and will be removed from
pandas in a future version. Use pandas.concat instead.
/users/jbenjami/.local/lib/python3.9/site-packages/plotnine/utils.py:371:
FutureWarning: The frame.append method is deprecated and will be removed from
pandas in a future version. Use pandas.concat instead.
```

1.9 Session information

```
[29]: session_info.show()
```

[29]: <IPython.core.display.HTML object>