main_exons

September 2, 2021

1 Enrichment and Overlap of PGC2+CLOZUK

[1]:
```
import re
import os, errno
import functools
import numpy as np
import pandas as pd
from plotnine import *
from pandas_plink import read_plink
from warnings import filterwarnings
from matplotlib.cbook import mplDeprecation
from scipy.stats import fisher_exact, binom_test

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

1.1 Config and Functions

[2]:
```
config = {
    'biomart_file': '../_h/biomart.csv',
    'phenotype_file': '/ceph/projects/v4_phase3_paper/inputs/phenotypes/_m/
 ↪merged_phenotypes.csv',
    'plink_file_prefix': '/ceph/projects/v4_phase3_paper/inputs/genotypes/_m/
 ↪LIBD_Brain_TopMed',
    'gwas_snp_file': '/ceph/projects/v4_phase3_paper/inputs/sz_gwas/pgc2_clozuk/
 ↪map_phase3/_m/libd_hg38_pgc2sz_snps.tsv',
    'exon_annotation': "../_h/exons.csv"
}

config_feature = {
    'de_file': '../../differential_expression/_m/exons/diffExpr_szVctl_full.
 ↪txt',
    'residual_expression_file': '../../differential_expression/_m/exons/
 ↪residualized_expression.tsv',
    'fastqtl_output_file': '../../eqtl/caudate/summary_table/_m/
 ↪Brainseq_LIBD_caudate_4features.signifpairs.txt.gz',
```

```
}

feature = "exons"
```

[3]:
```
@functools.lru_cache()
def get_exon_annot():
    return pd.read_csv(config['exon_annotation'])


@functools.lru_cache()
def feature_map(feature):
    return {"genes": "Gene", "transcripts": "Transcript",
            "exons": "Exon", "junctions": "Junction"}[feature]


@functools.lru_cache()
def get_de_df():
    """
    Load DE analysis
    """
    return pd.read_csv(config_feature['de_file'], sep='\t', index_col=0)


@functools.lru_cache()
def get_eqtl_df():
    eqtl_df = pd.read_csv(config_feature['fastqtl_output_file'], sep='\t')
    return eqtl_df[(eqtl_df["Type"] == feature_map(feature))]


@functools.lru_cache()
def get_gwas_snps():
    return pd.read_csv(config['gwas_snp_file'], sep='\t', index_col=0,␣
 ↪low_memory=False)


@functools.lru_cache()
def get_integration_df():
    return get_gwas_snps().merge(get_eqtl_df(), left_on='our_snp_id',␣
 ↪right_on='variant_id',
                                 suffixes=['_PGC2', '_eQTL'])\
                          .merge(get_de_df(), left_on='gene_id',␣
 ↪right_index=True)


@functools.lru_cache()
def get_residual_expression_df():
    return pd.read_csv(config_feature['residual_expression_file'],
```

```
                        sep='\t', index_col=0).transpose()


@functools.lru_cache()
def get_pheno_df():
    return pd.read_csv(config['phenotype_file'], index_col=0)
```

[4]:
```
def agree_direction(row):
    return [-1, 1][row['pgc2_a1_same_as_our_counted']] * np.sign(row['OR'] - 1)␣
 ↪* np.sign(row['slope']) * np.sign(row['t'])


def save_plot(p, fn):
    for ext in ['png', 'pdf', 'svg']:
        p.save(fn + '.' + ext)


def letter_snp(number, a0, a1):
    '''
    Example:
    letter_snp(0, 'A', 'G') is 'AA'
    letter_snp(1, 'A', 'G') is 'AG'
    letter_snp(2, 'A', 'G') is 'GG'

    '''
    if np.isnan(number):
        return np.nan

    if len(a0)==1 and len(a1)==1:
        sep = ''
    else:
        sep = ' '
    return sep.join(sorted([a0]*int(number) + [a1]*(2-int(number))))


def get_gwas_snp(snp_id):
    gwas = get_gwas_snps()
    r = gwas[gwas['our_snp_id']==snp_id]
    assert len(r) == 1
    return r
```

[5]:
```
@functools.lru_cache()
def get_expression_and_pheno_df():
    return pd.merge(get_pheno_df(), get_residual_expression_df(),␣
 ↪left_index=True, right_index=True)
```

```
@functools.lru_cache()
def get_plink_tuple():
    '''
    Usage: (bim, fam, bed) = get_plink_tuple()
    '''
    return read_plink(config['plink_file_prefix'])


@functools.lru_cache()
def subset_bed():
    """
    This subsets the bed and bim file and returns the new subsetted
    data with shared brain_ids.

    This is to speed up accessing the bed file.
    """
    (bim, fam, bed) = get_plink_tuple()
    brain_ids = list(set(get_expression_and_pheno_df()['BrNum']).
 ↪intersection(set(fam['fid'])))
    fam_pos = list(fam[(fam["fid"].isin(brain_ids))].
 ↪drop_duplicates(subset="fid").loc[:, 'i'])
    unique_snps = get_eqtl_df().variant_id.unique()
    snp_info = bim[(bim["snp"].isin(unique_snps))].copy()
    snp_pos = list(snp_info.loc[:, "i"])
    new_bed = bed[snp_pos].compute()[:,fam_pos]
    new_bim = bim[(bim["i"].isin(snp_pos))].reset_index(drop=True)
    new_bim['ii'] = new_bim.index
    return new_bed, new_bim, brain_ids


@functools.lru_cache()
def get_snp_df(snp_id):
    '''
    Returns a dataframe containing the genotype on snp snp_id.
    The allele count is the same as in the plink files.

    Example:
    get_snp_df('rs653953').head(5)

            rs653953_num rs653953_letter rs653953
    Br5168             0              GG    0\nGG
    Br2582             1              AG    1\nAG
    Br2378             1              AG    1\nAG
    Br5155             2              AA    2\nAA
    Br5182             2              AA    2\nAA
    '''
    bed, bim, brain_ids = subset_bed()
```

```
    snp_info = bim[bim['snp']==snp_id]
    snp_pos = snp_info.iloc[0]['ii']
    dfsnp = pd.DataFrame(bed[[snp_pos]], columns=brain_ids, index=[snp_id +␣
↪'_num']).transpose().dropna()
    my_letter_snp = functools.partial(letter_snp, a0=snp_info.iloc[0]['a0'],␣
↪a1=snp_info.iloc[0]['a1'])
    # the 2 - in next line is to workaround a possible bug in pandas_plink? a1␣
↪and a0 inverted
    dfsnp[[snp_id + '_num']] = 2 - dfsnp[[snp_id + '_num']].astype('int')
    dfsnp[snp_id + '_letter'] = dfsnp[snp_id + '_num'].apply(my_letter_snp)
    dfsnp[snp_id] = (dfsnp[snp_id + '_num'].astype('str') + '\n' +
                     dfsnp[snp_id + '_letter'].astype('str')).astype('category')
    return dfsnp


@functools.lru_cache()
def get_gwas_ordered_snp_df(snp_id):
    '''
    Returns a dataframe containing the genotype on snp snp_id.
    The allele count is the number of risk alleles according to GWAS.

    Example:
    get_gwas_ordered_snp_df('rs653953').head(5)

            rs653953_num rs653953_letter rs653953
    Br5168             2              GG    2\nGG
    Br2582             1              AG    1\nAG
    Br2378             1              AG    1\nAG
    Br5155             0              AA    0\nAA
    Br5182             0              AA    0\nAA
    '''
    pgc = get_gwas_snps()
    dfsnp = get_snp_df(snp_id).copy()
    gwas_snp = get_gwas_snp(snp_id)

    if gwas_snp['pgc2_a1_same_as_our_counted'].iloc[0]:
        if gwas_snp['OR'].iloc[0] > 1:
            pass
        else:
            dfsnp[[snp_id + '_num']] = 2 - dfsnp[[snp_id + '_num']]
    else:
        if gwas_snp['OR'].iloc[0] > 1:
            dfsnp[[snp_id + '_num']] = 2 - dfsnp[[snp_id + '_num']]
        else:
            pass

    dfsnp[snp_id] = (dfsnp[snp_id + '_num'].astype('str') + '\n' +
```

```
                    dfsnp[snp_id + '_letter'].astype('str')).astype('category')
    return dfsnp
```

[6]:
```
@functools.lru_cache()
def get_biomart_df():
    biomart = pd.read_csv(config['biomart_file'])
    biomart['description'] = biomart['description'].str.replace('\[Source.
 ↪*$','', regex=True)
    return biomart


def get_gene_symbol(gene_id, biomart=get_biomart_df()):
    ensge = re.sub('\..+$','', gene_id)
    ggg = biomart[biomart['ensembl_gene_id']==ensge]
    if ggg.shape[0]==0:
        return '', ''
    gs = ggg['external_gene_name'].values[0]
    de = ggg['description'].values[0]
    if type(de)!=str:
        de = ''
    de = re.sub('\[Source:.*$','',de)
    return gs, de


@functools.lru_cache()
def get_risk_allele(snp_id):
    gwas_snp = get_gwas_snp(snp_id)
    if gwas_snp['OR'].iloc[0] > 1:
        ra = gwas_snp['A1'].iloc[0]
    else:
        ra = gwas_snp['A2'].iloc[0]
    return ra
```

[7]:
```
def get_snp_gene_pheno_df(snp_id, gene_id, snp_df_func):
    pheno_columns = list(get_pheno_df().columns)
    expr_df = get_expression_and_pheno_df()[pheno_columns + [gene_id]]
    snp_df =  snp_df_func(snp_id)
    return expr_df.merge(snp_df, left_on='BrNum', right_index=True)


def simple_snp_expression_pheno_plot_impl(snp_id, gene_id, snp_df_func,␣
 ↪pheno_var):
    df = get_snp_gene_pheno_df(snp_id, gene_id, snp_df_func)
    df['Dx'] = df.Dx.astype('category').cat.rename_categories({'Control':␣
 ↪'CTL', 'Schizo': 'SZ'})
    y0 = df[gene_id].quantile(.01) - 0.26
    y1 = df[gene_id].quantile(.99) + 0.26
```

```
    pjd = position_jitterdodge(jitter_width=0.27)
    p = ggplot(df, aes(x=snp_id, y=gene_id, fill=pheno_var)) \
    + geom_boxplot(alpha=0.4, outlier_alpha=0) \
    + geom_jitter(position=pjd, stroke=0, alpha=0.6) + ylim(y0, y1) \
    + labs(y='Residualized expression', fill='Diagnosis') \
    + theme_bw(base_size=20)\
    + theme(legend_title=element_text(face='bold'),
            panel_grid_major=element_blank(),
            panel_grid_minor=element_blank())
    return p


def simple_gwas_ordered_snp_expression_pheno_plot(snp_id, gene_id, pheno_var):
    return simple_snp_expression_pheno_plot_impl(snp_id, gene_id,␣
 ↪get_gwas_ordered_snp_df, pheno_var)
```

[8]:
```
def exon_annotation(gene_id):
    return get_exon_annot()[get_exon_annot().brainseq_exon_id == gene_id].
 ↪exon_id.values[0]


def gwas_annotation(snp_id):
    return 'SZ GWAS pvalue: %.1e' % get_gwas_snp(snp_id).iloc[0]['P']


def eqtl_annotation(snp_id, gene_id):
    r = get_eqtl_df()[(get_eqtl_df()['variant_id']==snp_id) &
                      (get_eqtl_df()['gene_id']==gene_id)]
    assert len(r)==1
    return 'eQTL nominal p-value: %.1e' % r.iloc[0]['pval_nominal']


def de_annotation(gene_id):
    de_df = get_de_df()
    de_df['Feature'] = de_df.index
    g = de_df[(de_df['Feature'] == gene_id)]
    return 'DE adj.P.Val: %.3f' % g.iloc[0]['adj.P.Val']


def risk_allele_annotation(snp_id):
    return 'SZ risk allele: %s' % get_risk_allele(snp_id)


def gwas_annotated_eqtl_pheno_plot(snp_id, gene_id, pheno_var):
    p = simple_gwas_ordered_snp_expression_pheno_plot(snp_id, gene_id,␣
 ↪pheno_var)
    de_df = get_de_df()
```

```
    de_df['Feature'] = de_df.index
    de_df = de_df[(de_df['Feature'] == gene_id)]
    gene_symbol, gene_description = get_gene_symbol(de_df.iloc[0]['gencodeID'])
    exon_id = exon_annotation(gene_id)
    title ="\n".join([gene_symbol,
                      exon_id,
                      gwas_annotation(snp_id),
                      risk_allele_annotation(snp_id),
                      eqtl_annotation(snp_id, gene_id),
                      de_annotation(gene_id)])
    p += ggtitle(title)
    return p
```

1.2 Exons

[9]:
```
try:
    os.makedirs(feature)
except OSError as e:
    if e.errno != errno.EEXIST:
        raise
```

1.2.1 Enrichment

Integrate DEG with PGC2+CLOZUK SNPs

[10]:
```
dft = get_integration_df()
dft.shape
```

```
/home/jbenja13/.local/lib/python3.9/site-packages/numpy/lib/arraysetops.py:583:
FutureWarning: elementwise comparison failed; returning scalar instead, but in
the future will perform elementwise comparison
```

[10]:
```
(3650186, 52)
```

[11]:
```
agreement = {1: 'Yes', -1: 'No', 0: 0}
dft['agree_direction'] = dft.apply(agree_direction, axis=1)
dft.agree_direction = [agreement[item] for item in dft['agree_direction']]

table =  [[np.sum((dft['P']<5e-8) & ((dft['adj.P.Val']<.05))),
           np.sum((dft['P']<5e-8) & ((dft['adj.P.Val']>=.05)))],
          [np.sum((dft['P']>=5e-8) & ((dft['adj.P.Val']<.05))),
           np.sum((dft['P']>=5e-8) & ((dft['adj.P.Val']>=.05)))]]
print(table)
fisher_exact(table)
```

```
[[3962, 67556], [256601, 3322067]]
```

[11]:
```
(0.7592775985025325, 1.0709599924360984e-67)
```

[12]:
```
dft1 = dft[(dft['P']<5e-8) & ((dft['adj.P.Val']<.05))]
df = dft1.groupby('agree_direction').size().reset_index()
df
```

[12]:

	agree_direction	0
0	No	574
1	Yes	3388

[13]:
```
binom_test(df[0].iloc[1], df[0].sum())
```

[13]:
```
0.0
```

[14]:
```
dft2 = dft[(dft['P']<=5e-8) & (dft['adj.P.Val'] < 0.05)].copy()
dft2['risk_allele'] = dft2['our_snp_id'].apply(get_risk_allele)
```

[15]:
```
direction = {-1: 'Down', 1: 'Up'}
boolean_conv = {True: 1, False: -1}
dft2.pgc2_a1_same_as_our_counted = [boolean_conv[item] for item in␣
 ↪dft2['pgc2_a1_same_as_our_counted']]
dft2['eqtl_gwas_dir'] = [direction[item] for item in np.
 ↪sign(dft2['pgc2_a1_same_as_our_counted']) * np.sign(dft2['slope']) * np.
 ↪sign(dft2['OR'] - 1)]
dft2['de_dir'] = [direction[item] for item in np.sign(dft2['t'])]
dft2['eqtl_slope'] = np.sign(dft2['pgc2_a1_same_as_our_counted']) * np.
 ↪sign(dft2['OR'] - 1) * dft2['slope']
dft2 = dft2[['gene_id', 'Symbol', 'variant_id', 'A1', 'A2', 'risk_allele', 'OR',
             'P', 'pval_nominal', 'adj.P.Val', 'logFC', 't', 'eqtl_slope',
             'de_dir', 'eqtl_gwas_dir', 'agree_direction']]
dft2['Symbol'].fillna(dft2['gene_id'], inplace=True)
dft2.to_csv('%s/integration_by_symbol.txt' % feature, sep='\t', index=False)
```

[16]:
```
df2 = dft2.groupby(['Symbol']).first().reset_index().sort_values('P')
df2.groupby('agree_direction').size()
```

[16]:
```
agree_direction
No     11
Yes    14
dtype: int64
```

[17]:
```
df2.set_index('Symbol').rename(columns={'t': 'de_t', 'P': 'GWAS_P',␣
 ↪'pval_nominal': 'eQTL_pvalue',
                                        'adj.P.Val': 'de_FDR'})
```

[17]:

Symbol	gene_id	variant_id	A1	A2	risk_allele	OR	\
HCG4	e379384	chr6:29292654:C:T	C	T	C	1.26140	
ZNF391	e378375	chr6:27003116:C:A	C	A	C	1.19440	

FLOT1	e380557	chr6:30261490:G:C	G	C	G	1.23800	
BRD2	e386363	chr6:32634921:C:T	C	T	C	1.17320	
ZSCAN26	e378558	chr6:27772887:C:T	C	T	C	1.10460	
PPP1R10	e380196	chr6:30856674:T:C	T	C	T	1.12860	
HCG11	e378174	chr6:26466161:G:A	G	A	A	0.91432	
ATF6B	e385196	chr6:31636233:C:G	C	G	G	0.92349	
NGEF	e193333	chr2:232920039:T:G	T	G	G	0.92446	
PLCH2	e4214	chr1:2440958:A:G	A	G	G	0.92873	
e805477	e805477	chr14:103551456:G:A	G	A	A	0.93503	
SLC35G2	e251472	chr3:136789166:C:A	C	A	A	0.94040	
ZNF204P	e378369	chr6:27399042:C:A	C	A	C	1.06050	
CNNM2	e598887	chr10:102825368:C:A	C	A	C	1.06040	
PCCB	e251235	chr3:136088767:G:T	G	T	T	0.94493	
ZFYVE21	e805625	chr14:103541822:C:T	C	T	T	0.93994	
HIRIP3	e880446	chr16:29959536:C:G	C	G	C	1.05670	
PPM1M	e226757	chr3:52253452:G:T	G	T	G	1.05600	
ZC3H7B	e1136648	chr22:41357599:G:A	G	A	A	0.94102	
REEP2	e353080	chr5:138362610:C:T	C	T	C	1.05600	
IP6K3	e387950	chr6:33726302:C:T	C	T	C	1.07170	
B3GAT1	e678063	chr11:134426490:C:T	C	T	C	1.05480	
SLC7A6	e897871	chr16:68251944:A:G	A	G	G	0.93292	
ZNF14	e1037535	chr19:19623068:T:C	T	C	T	1.06130	
LINC01470	e361367	chr5:152550275:T:C	T	C	T	1.06120	

Symbol	GWAS_P	eQTL_pvalue	de_FDR	logFC	de_t	\
HCG4	6.890000e-39	4.889500e-05	1.888890e-02	0.225849	3.403813	
ZNF391	1.330000e-30	8.931290e-05	3.050985e-02	0.089084	3.198447	
FLOT1	4.410000e-27	2.234560e-04	4.343551e-02	-0.056802	-3.035928	
BRD2	1.050000e-22	1.767730e-04	4.566092e-02	-0.055920	-3.012771	
ZSCAN26	1.180000e-18	4.878850e-04	1.907918e-02	-0.084425	-3.399939	
PPP1R10	2.320000e-17	2.833910e-04	3.267149e-02	-0.097099	-3.167716	
HCG11	1.020000e-14	2.926190e-06	1.114014e-03	0.123153	4.437634	
ATF6B	1.580000e-13	5.443640e-05	4.105628e-02	-0.077310	-3.062621	
NGEF	2.030000e-13	3.210140e-05	3.934485e-02	0.094485	3.083163	
PLCH2	4.630000e-11	2.092640e-08	2.092699e-03	-0.183916	-4.228084	
e805477	2.500000e-10	2.159220e-04	4.326236e-02	0.088201	3.037782	
SLC35G2	6.440000e-10	2.369150e-04	1.775019e-02	0.087134	3.429320	
ZNF204P	6.470000e-10	2.912170e-04	2.730366e-02	0.101791	3.247301	
CNNM2	1.120000e-09	1.275560e-10	1.824329e-03	0.082170	4.275115	
PCCB	5.050000e-09	2.777040e-04	4.550772e-02	-0.086627	-3.014289	
ZFYVE21	6.910000e-09	4.960450e-05	1.172012e-03	0.120871	4.421534	
HIRIP3	1.190000e-08	5.943010e-05	1.852549e-02	-0.086915	-3.411509	
PPM1M	1.350000e-08	2.619140e-04	6.255691e-03	-0.112520	-3.838539	
ZC3H7B	1.760000e-08	3.336000e-05	2.460607e-02	-0.069774	-3.293462	
REEP2	1.860000e-08	2.256820e-07	9.024244e-08	0.146930	6.740507	
IP6K3	2.460000e-08	5.035440e-06	2.516434e-02	-0.206644	-3.283807	

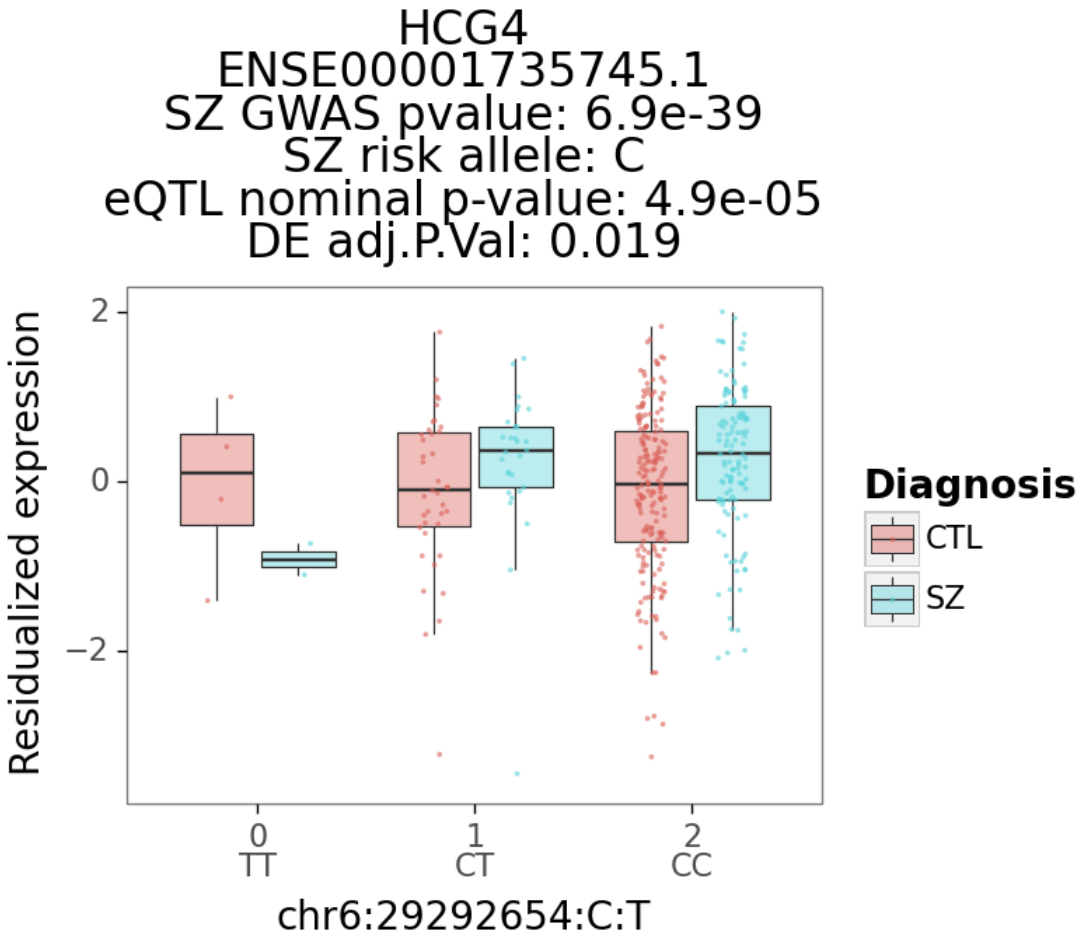
```
B3GAT1     2.980000e-08  2.428260e-05  5.232855e-03 -0.095884 -3.902576
SLC7A6     3.560000e-08  1.304730e-04  2.399729e-02  0.074993  3.304797
ZNF14      4.300000e-08  1.046650e-06  1.193200e-02  0.089690  3.587360
LINC01470  4.470000e-08  1.436330e-11  7.415818e-03  0.475060  3.772128
```

Symbol	eqtl_slope	de_dir	eqtl_gwas_dir	agree_direction
HCG4	0.376665	Up	Up	Yes
ZNF391	-0.248239	Up	Down	No
FLOT1	-0.181652	Down	Down	Yes
BRD2	-0.263443	Down	Down	Yes
ZSCAN26	-0.208406	Down	Down	Yes
PPP1R10	0.197282	Down	Up	No
HCG11	0.242904	Up	Up	Yes
ATF6B	0.205829	Down	Up	No
NGEF	-0.124147	Up	Down	No
PLCH2	-0.177917	Down	Down	Yes
e805477	0.160045	Up	Up	Yes
SLC35G2	-0.145741	Up	Down	No
ZNF204P	0.118762	Up	Up	Yes
CNNM2	-0.172984	Up	Down	No
PCCB	-0.175844	Down	Down	Yes
ZFYVE21	0.229127	Up	Up	Yes
HIRIP3	0.207663	Down	Up	No
PPM1M	-0.126091	Down	Down	Yes
ZC3H7B	0.185178	Down	Up	No
REEP2	0.162459	Up	Up	Yes
IP6K3	0.184969	Down	Up	No
B3GAT1	0.115385	Down	Up	No
SLC7A6	-0.155408	Up	Down	No
ZNF14	0.248788	Up	Up	Yes
LINC01470	0.400474	Up	Up	Yes

1.2.2 Plot with PGC2 risk allele

[18]:
```
for xx in range(df2.shape[0]):
    gg = gwas_annotated_eqtl_pheno_plot(df2.iloc[xx, :].variant_id, df2.
 ↪iloc[xx, :].gene_id, 'Dx')
    print(gg)
    label = '%s/eqtl_gwas_%s' % (feature, df2.iloc[xx, :].Symbol)
    save_plot(gg, label)
```

```
Mapping files: 100%|      | 3/3 [00:25<00:00,  8.55s/it]
```




```
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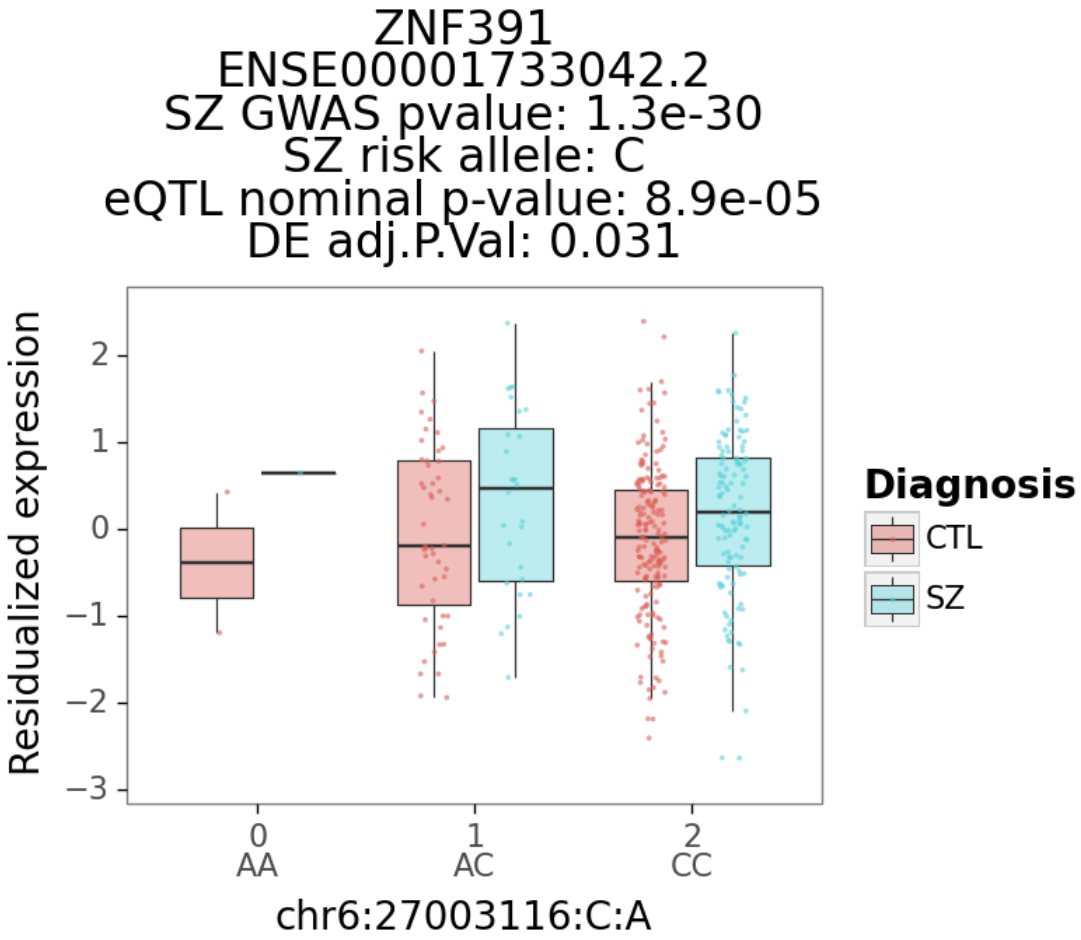
```




```
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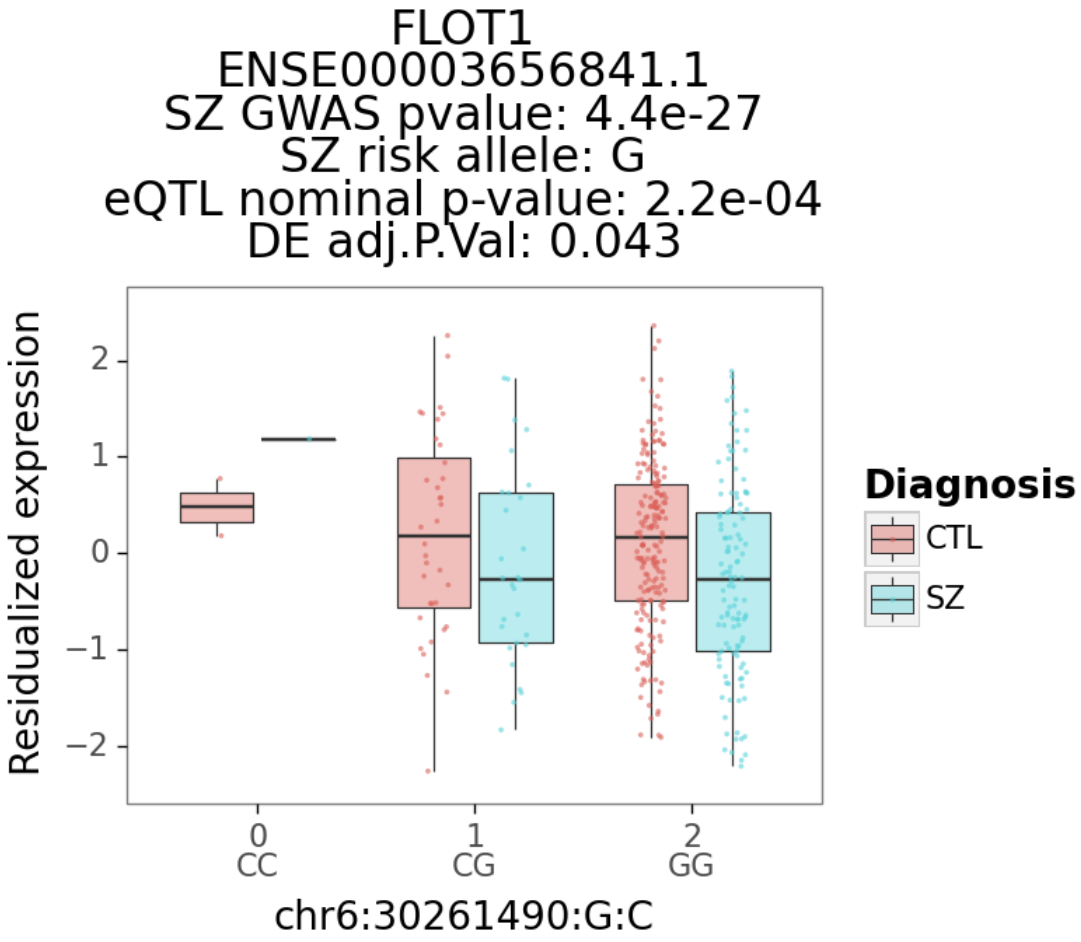


```
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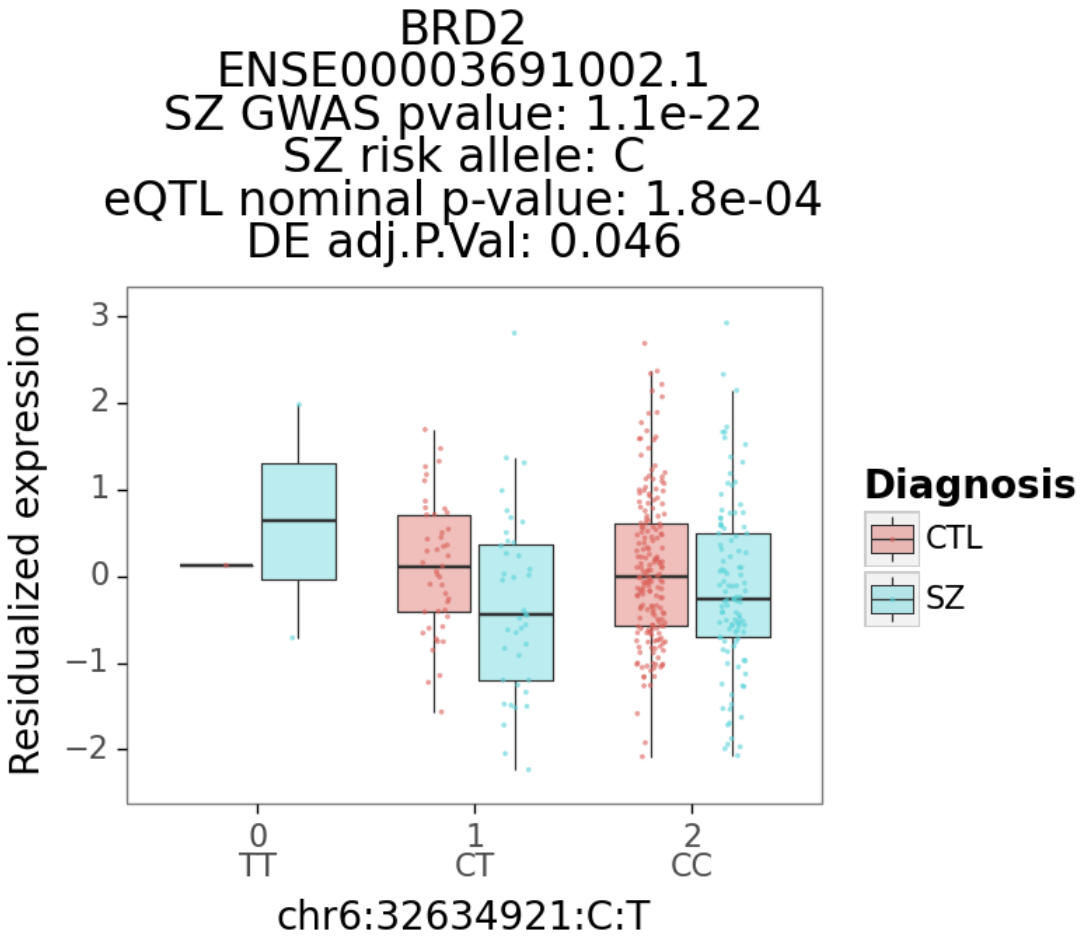


```
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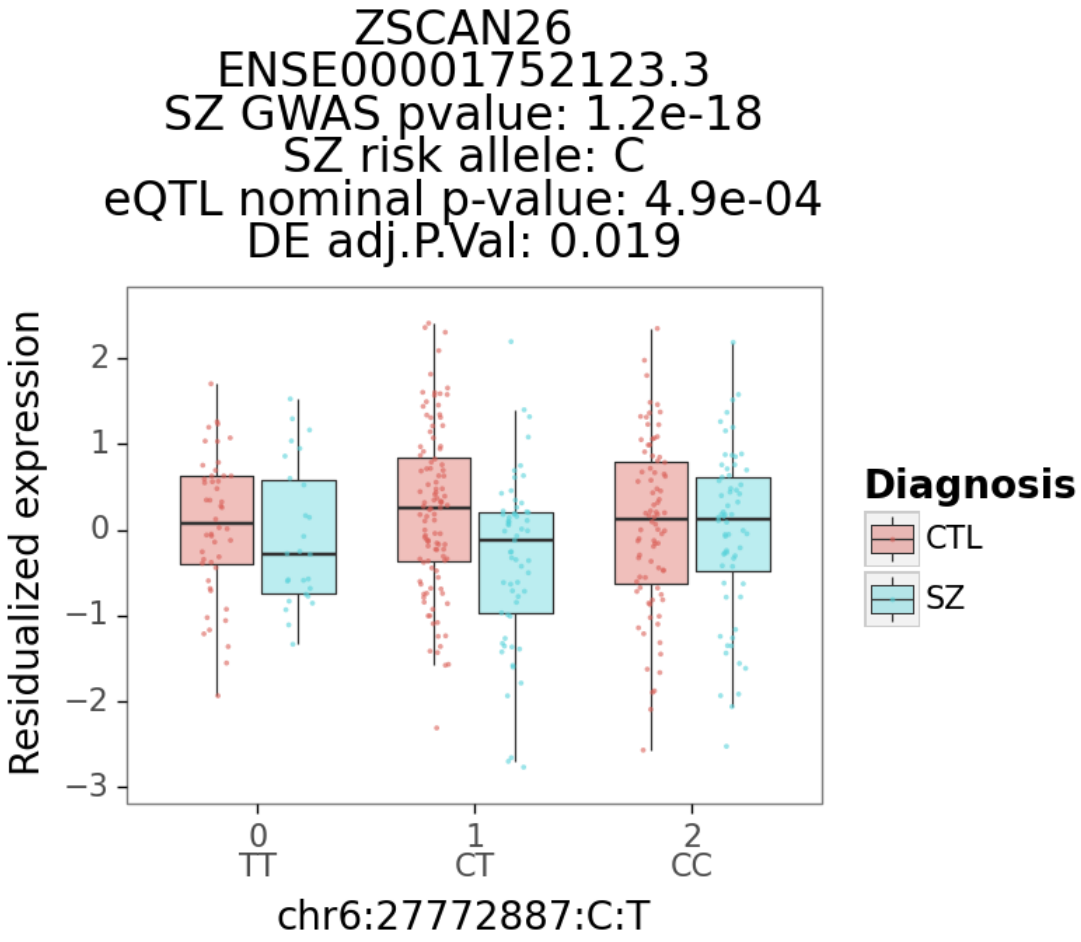


```
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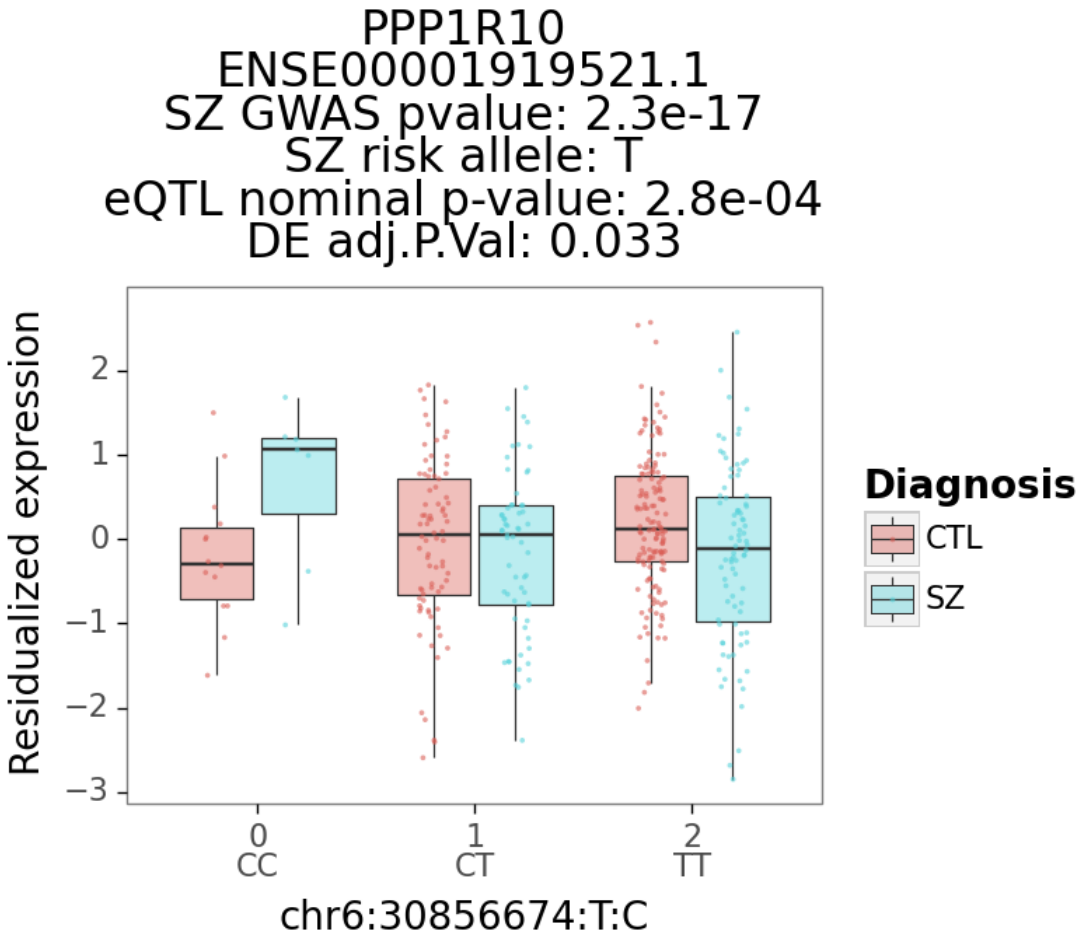


```
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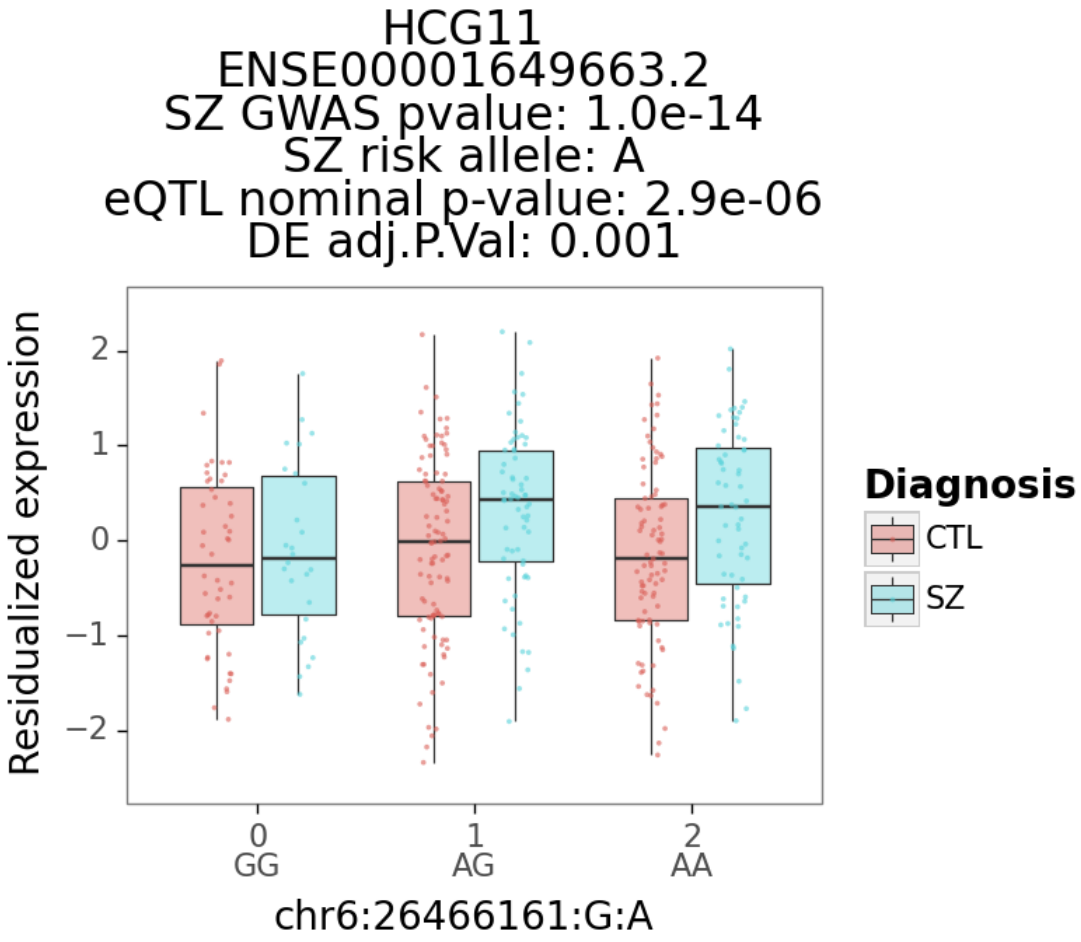


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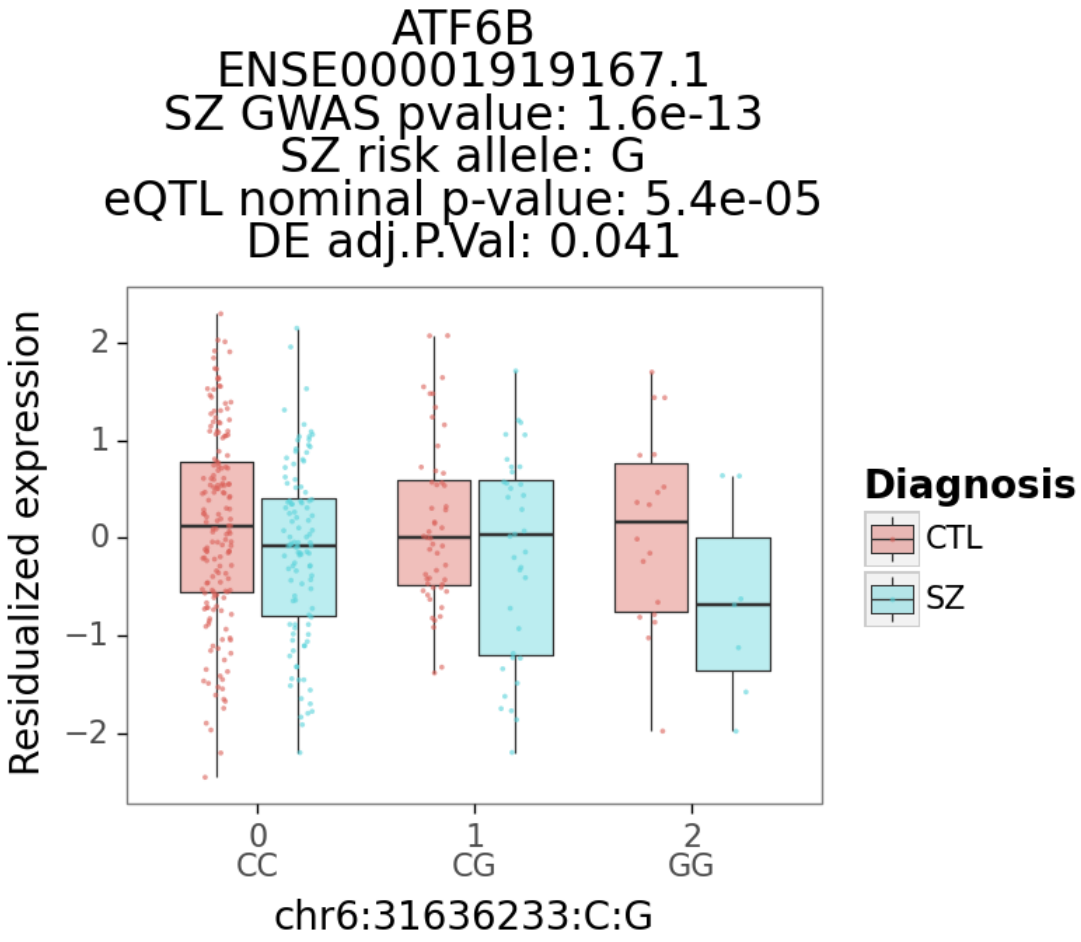


```
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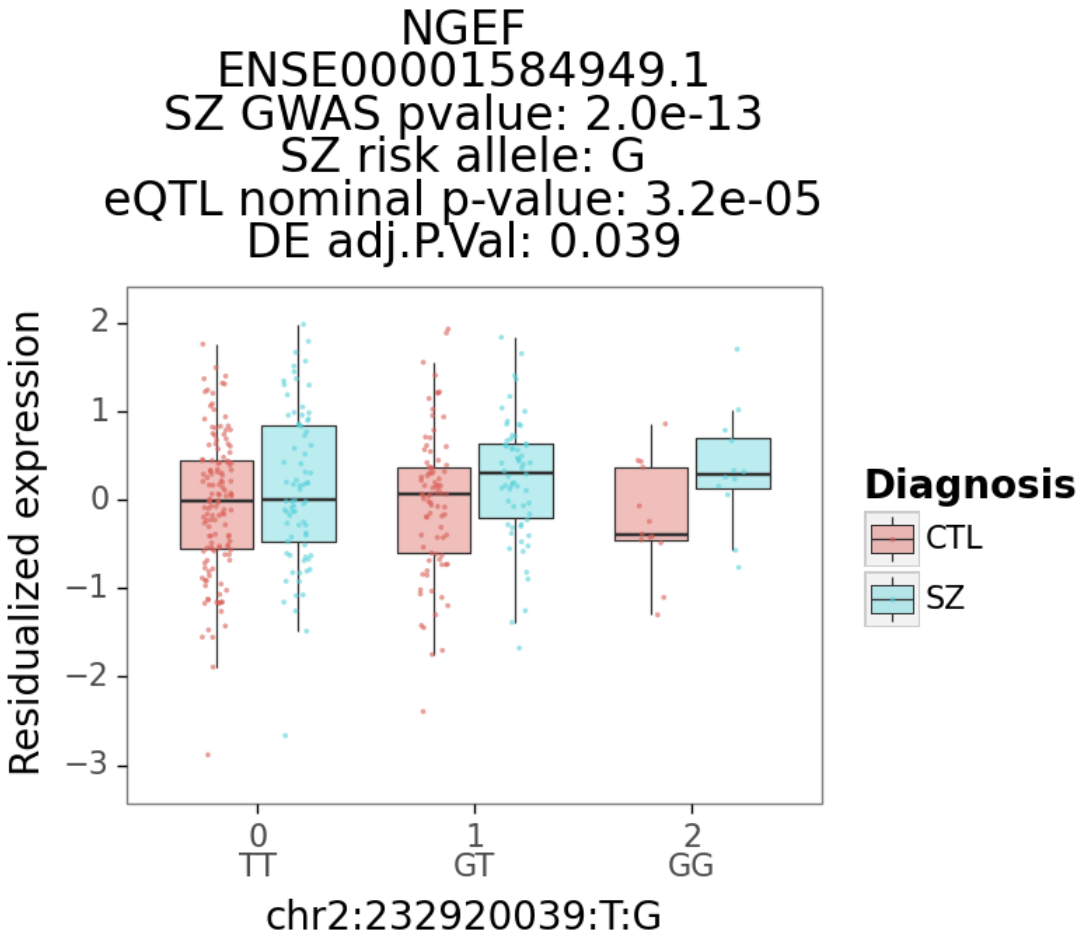


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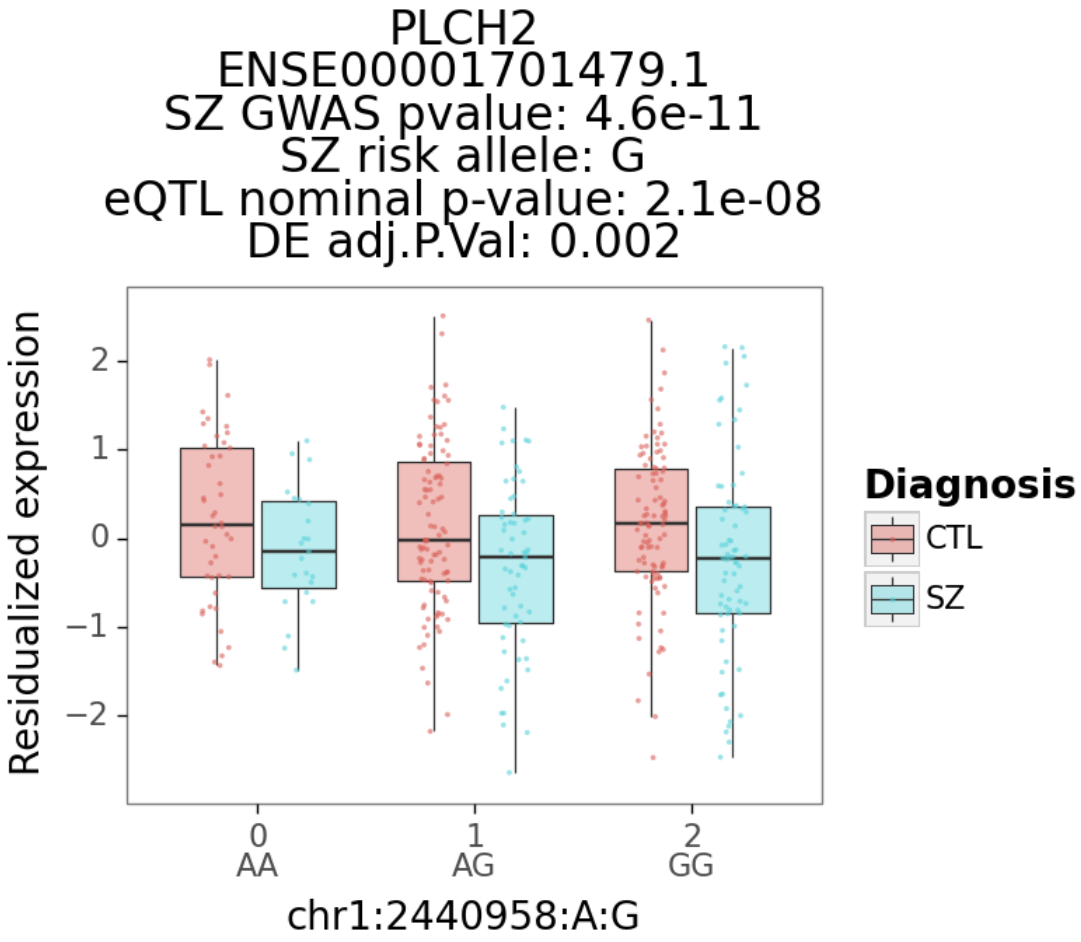


```
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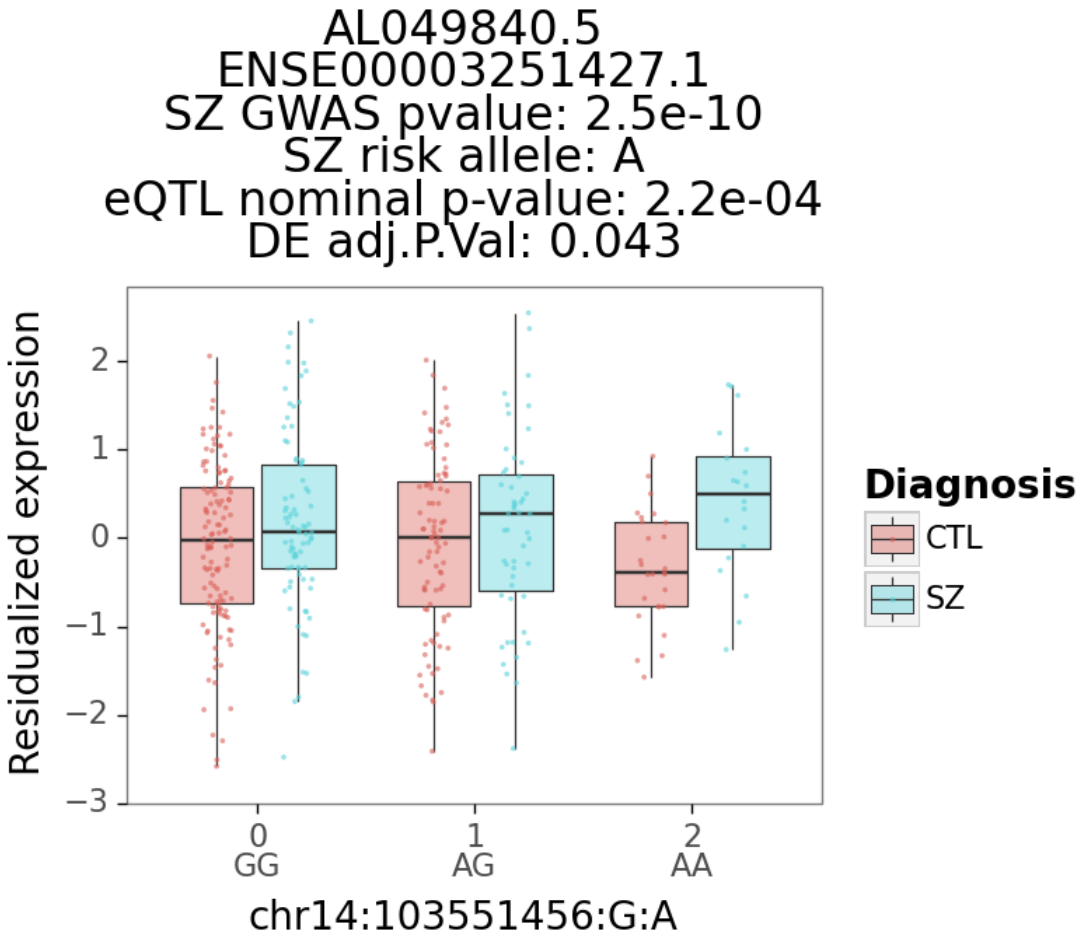

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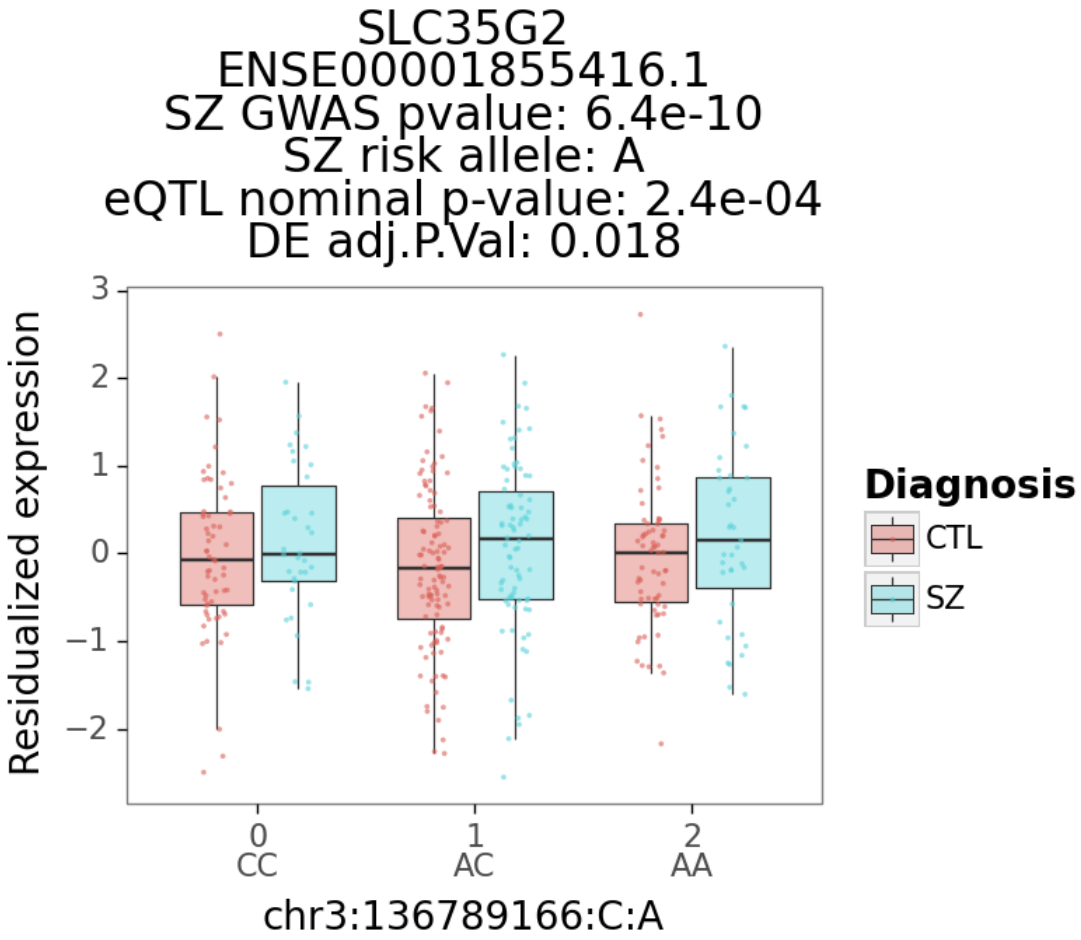


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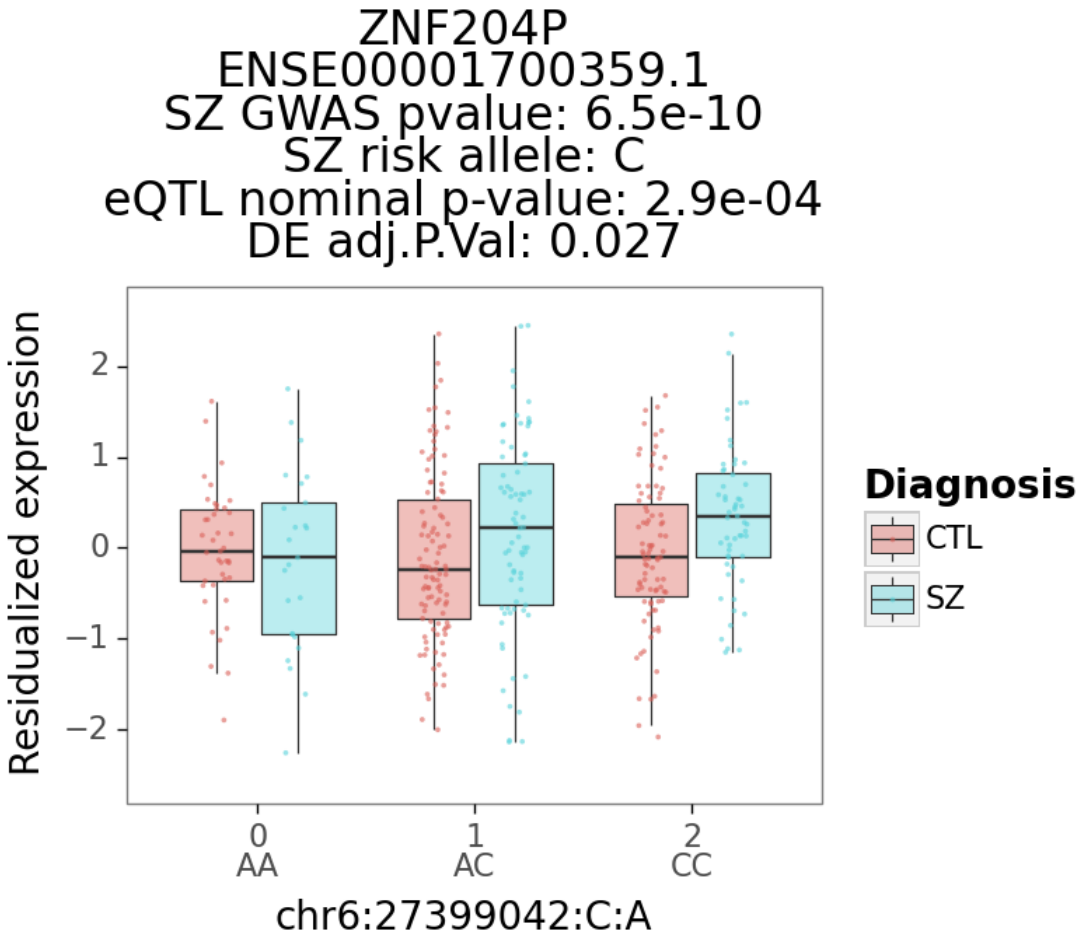


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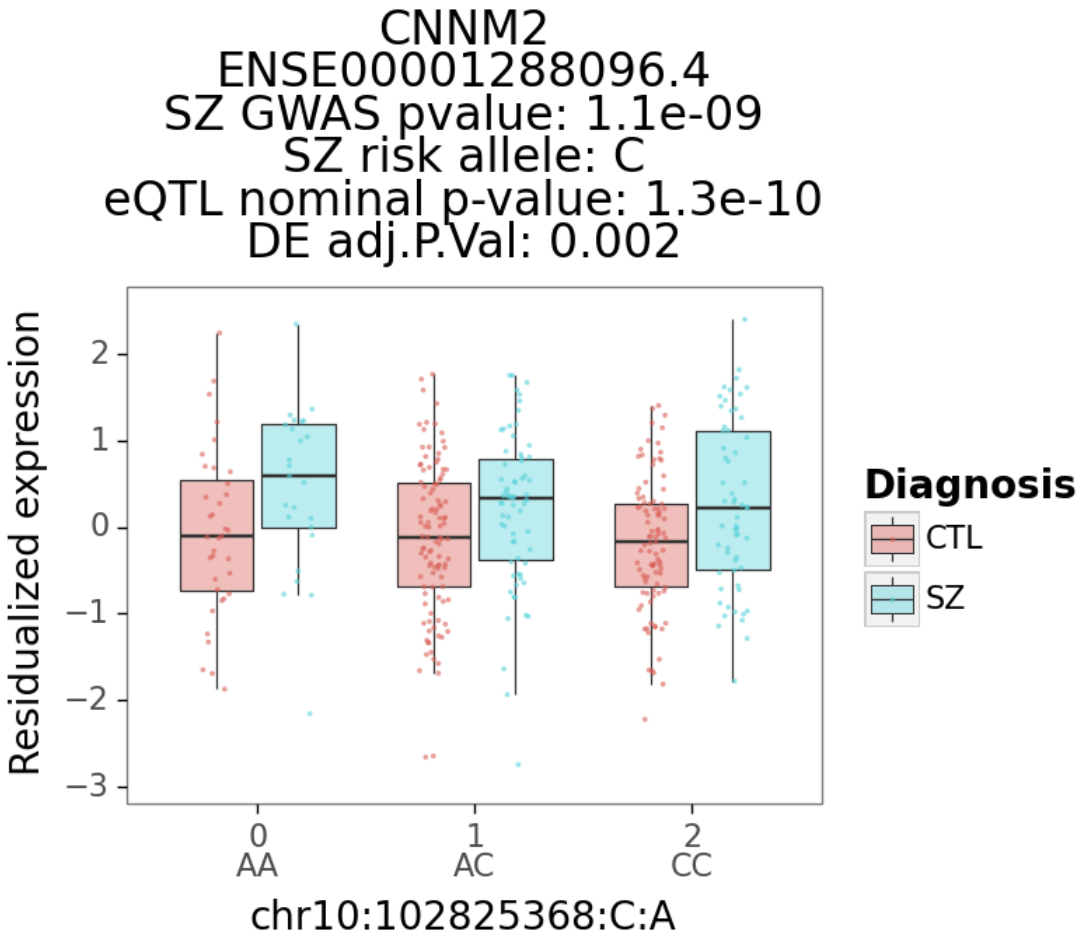


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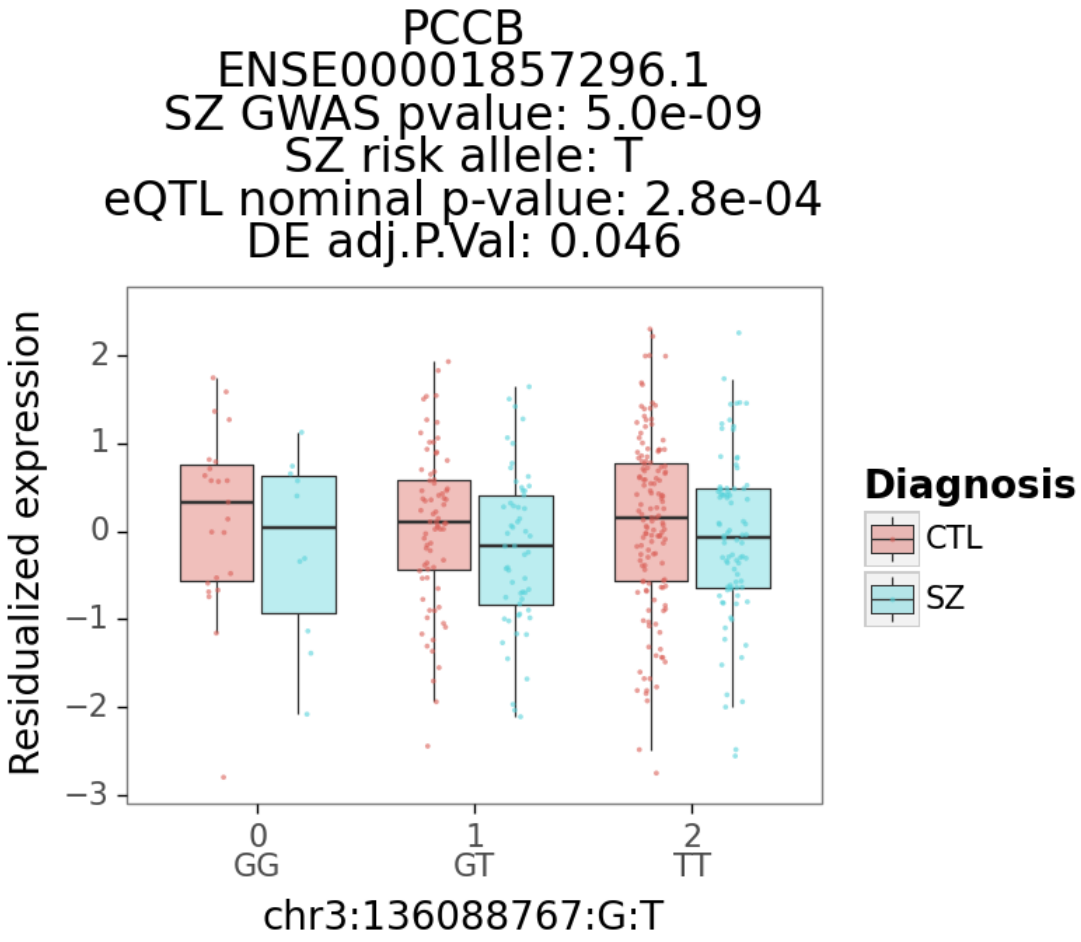


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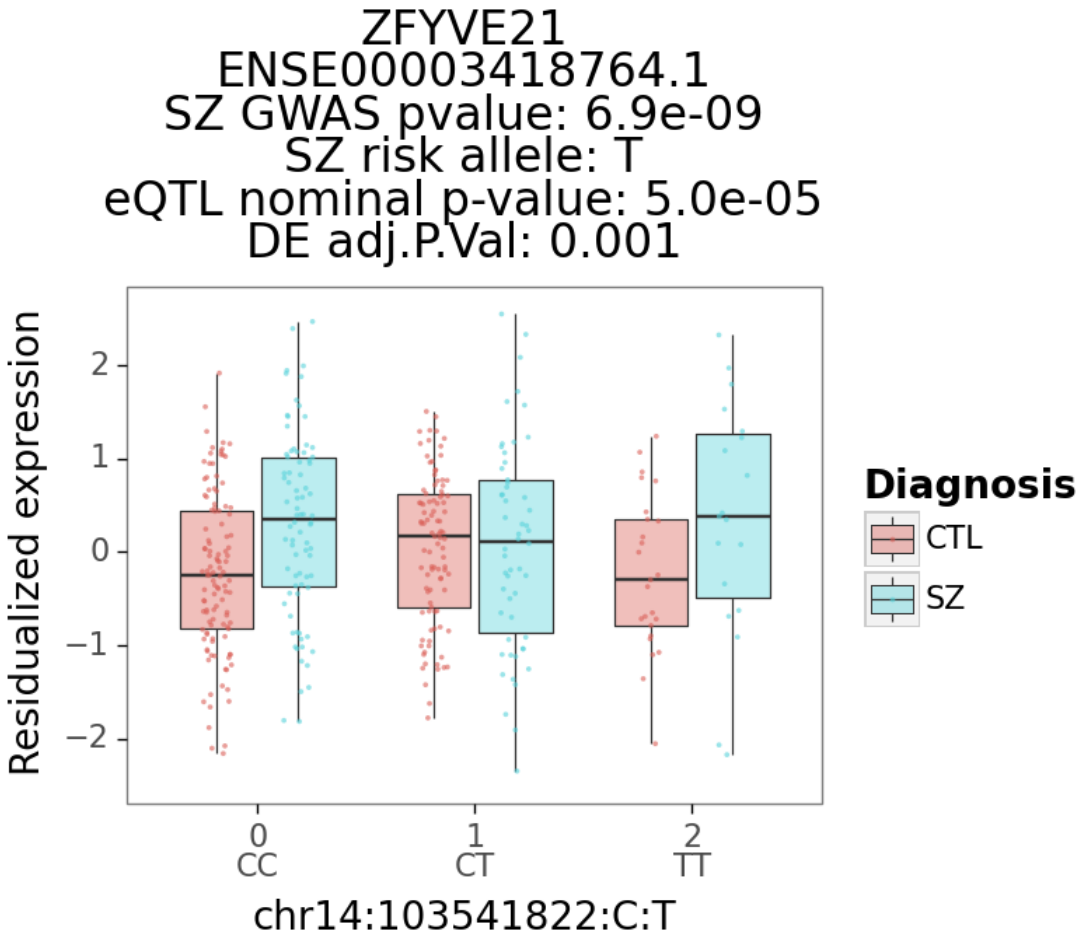


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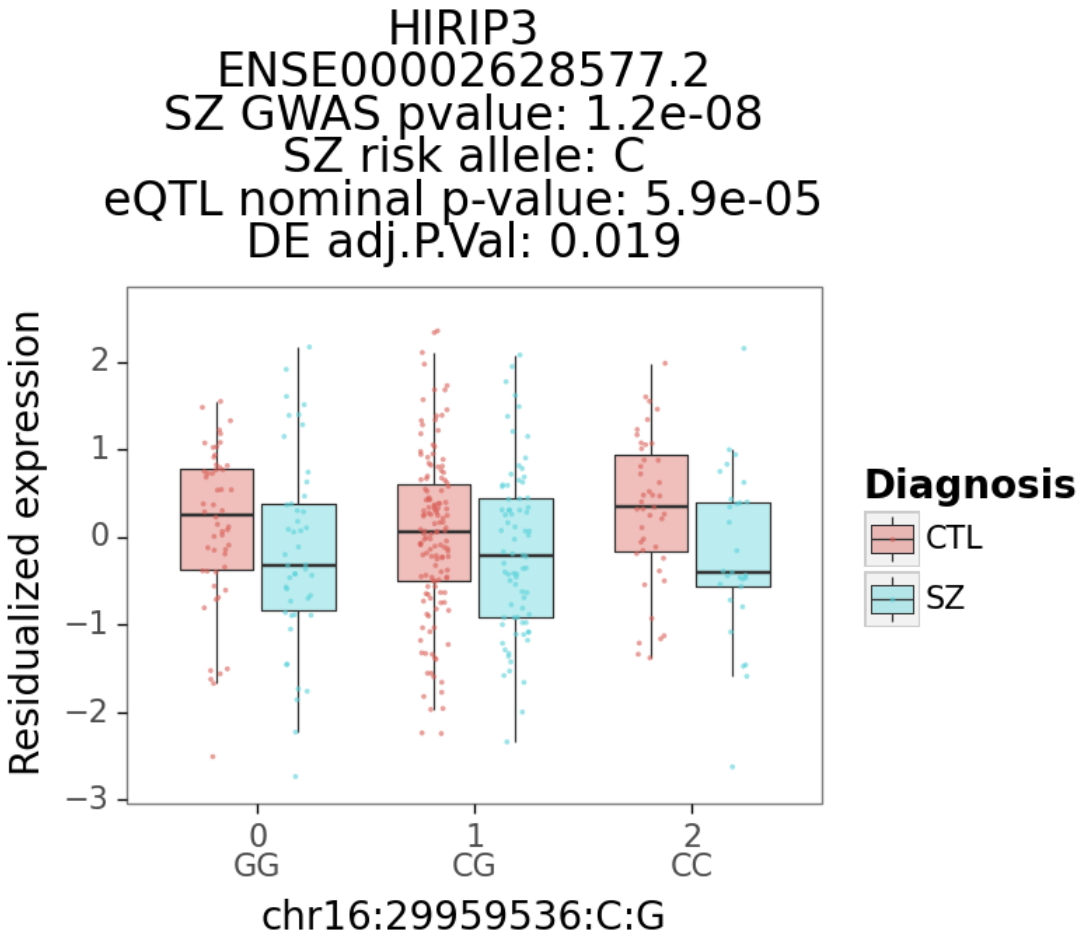


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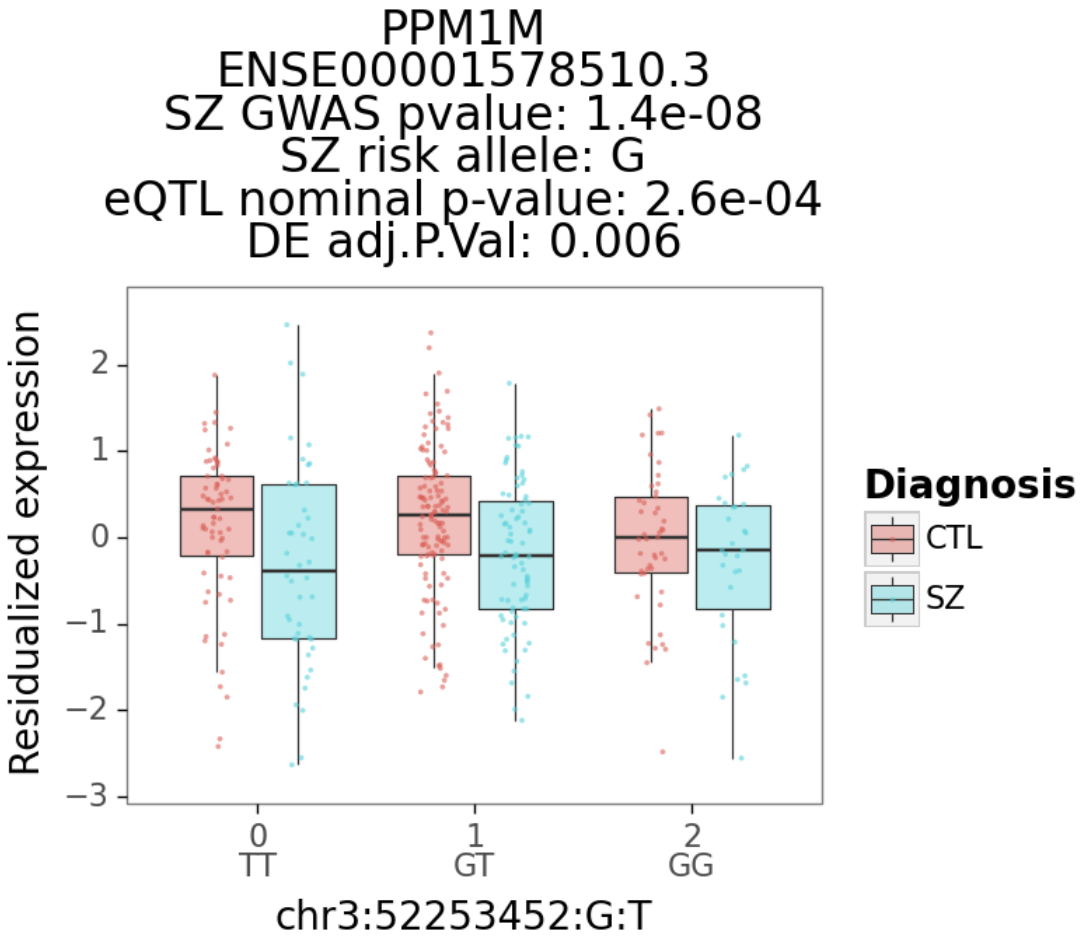


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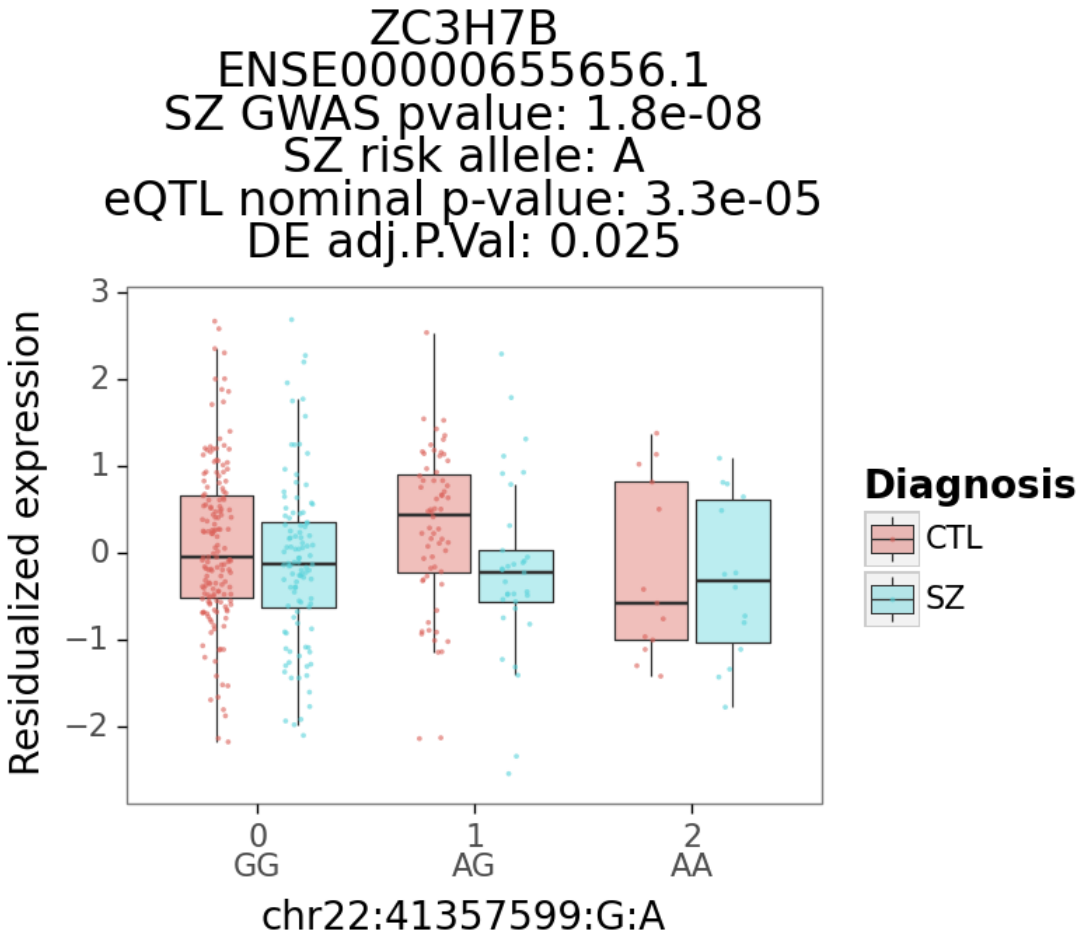


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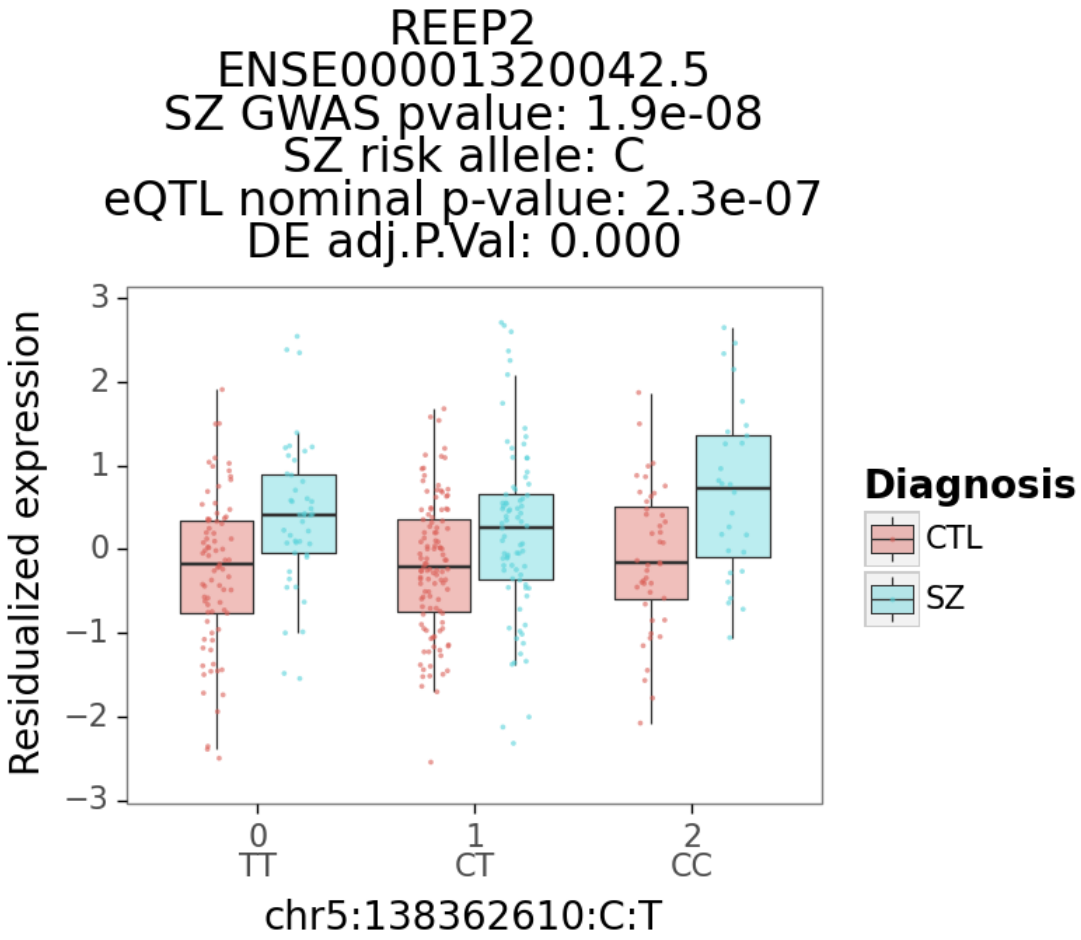


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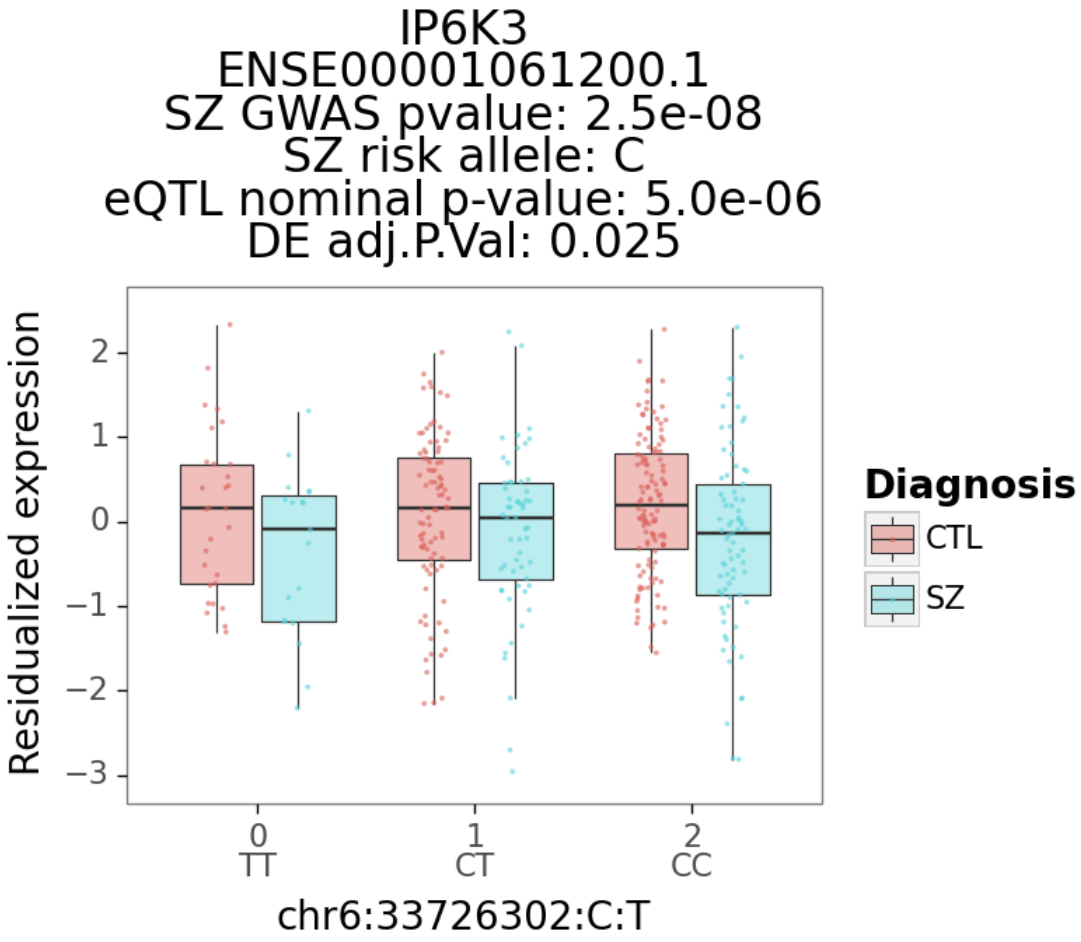


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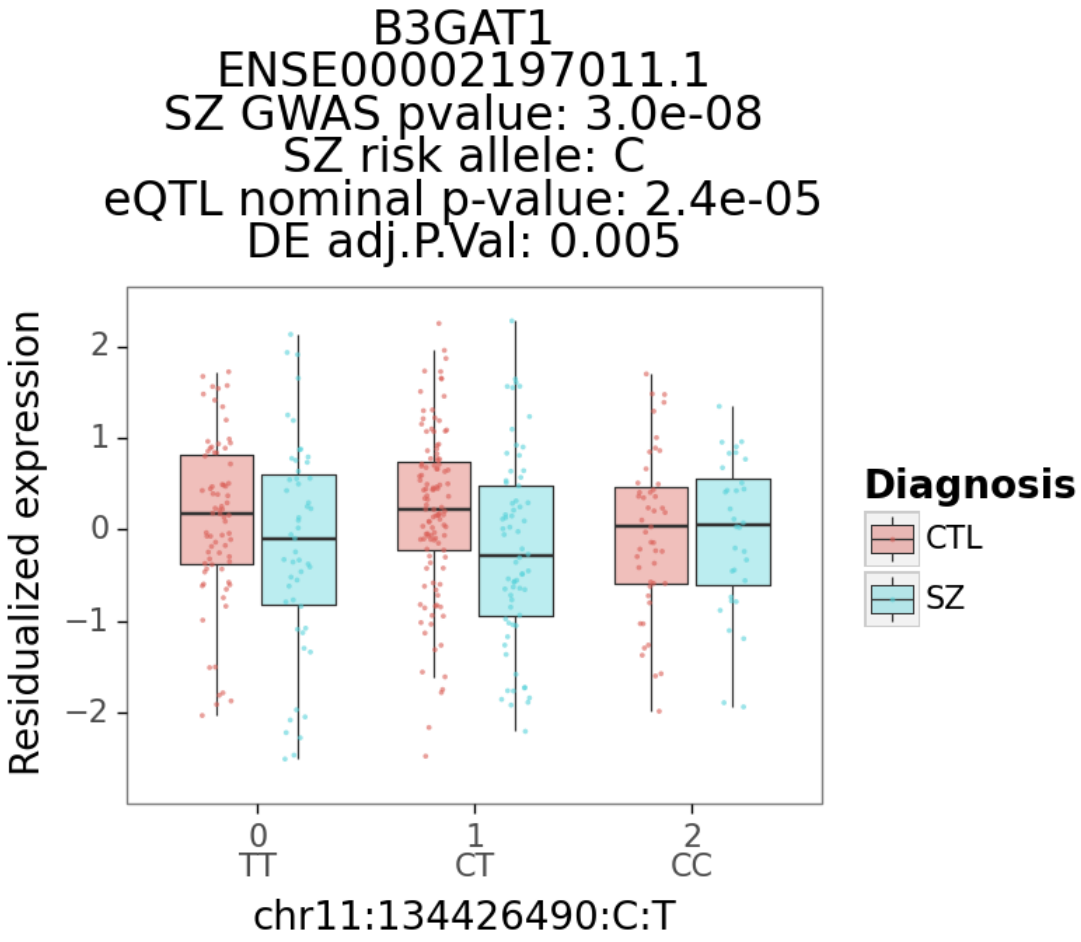


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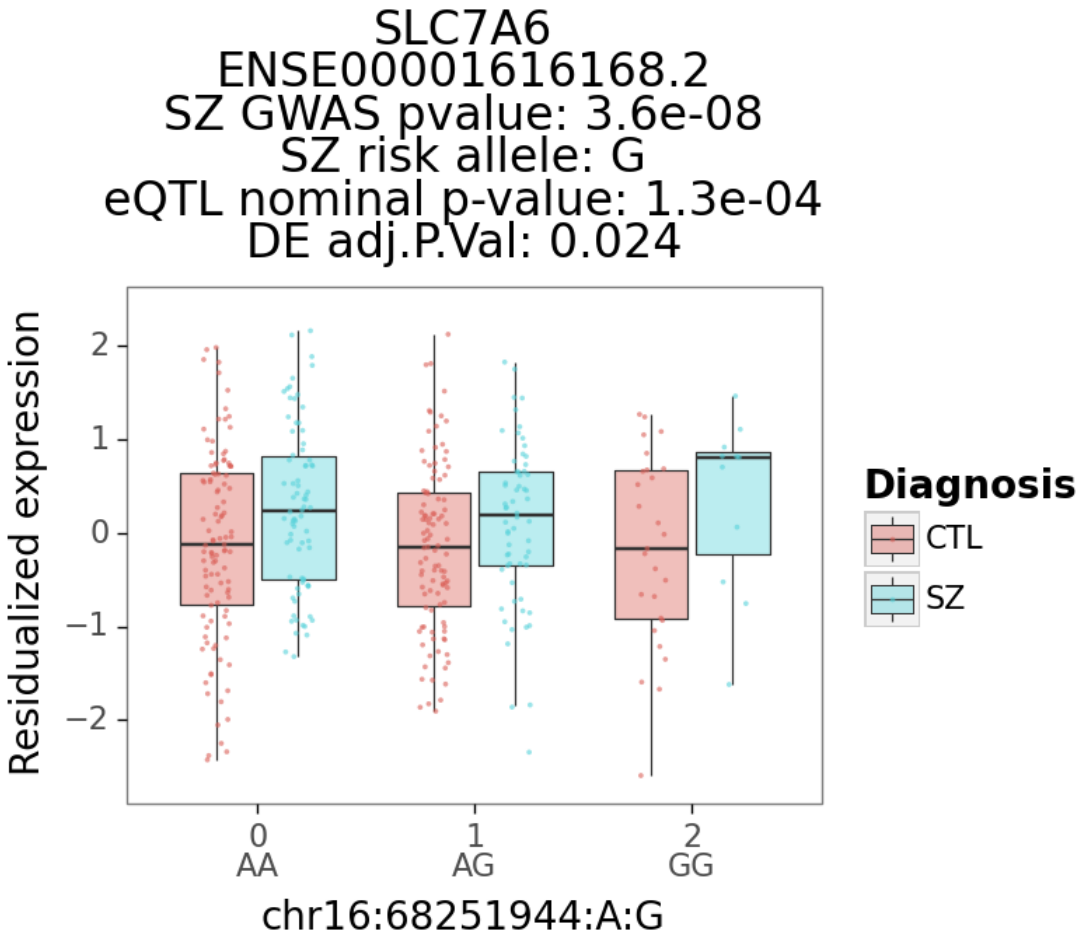


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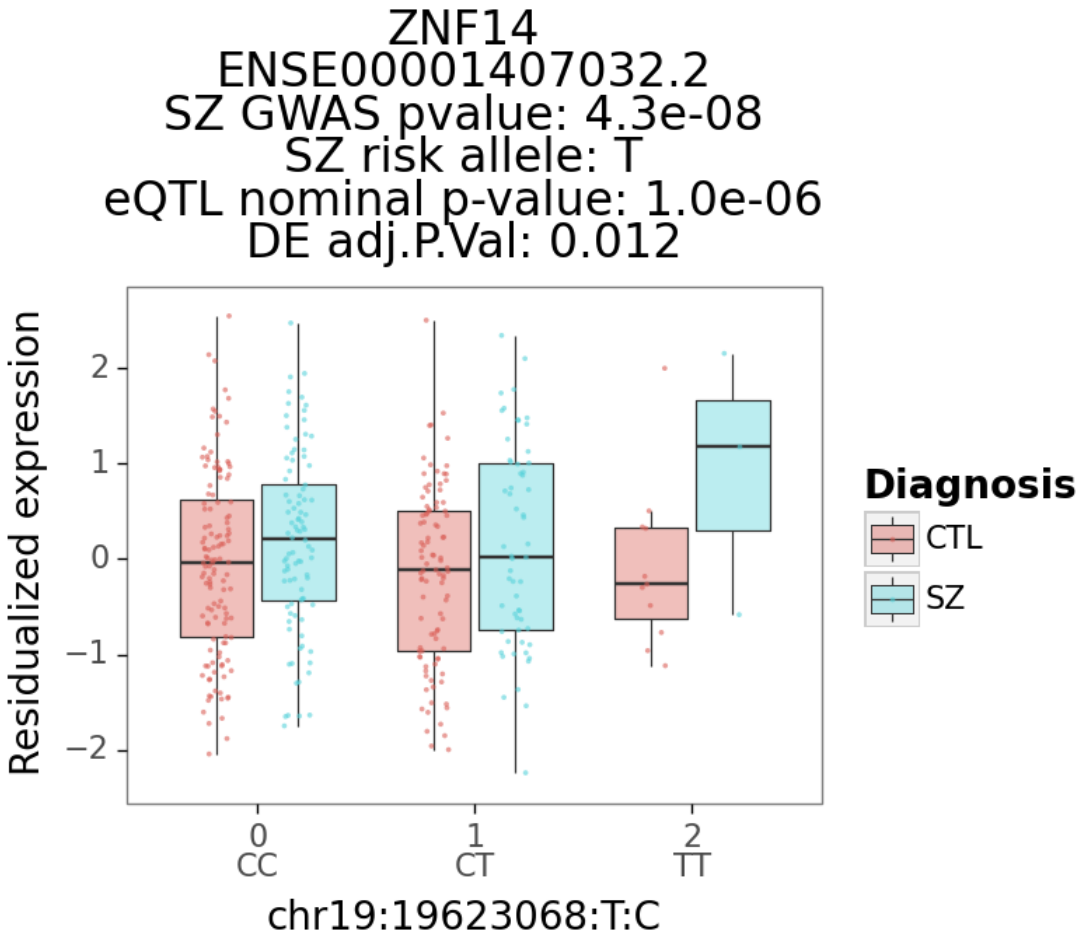


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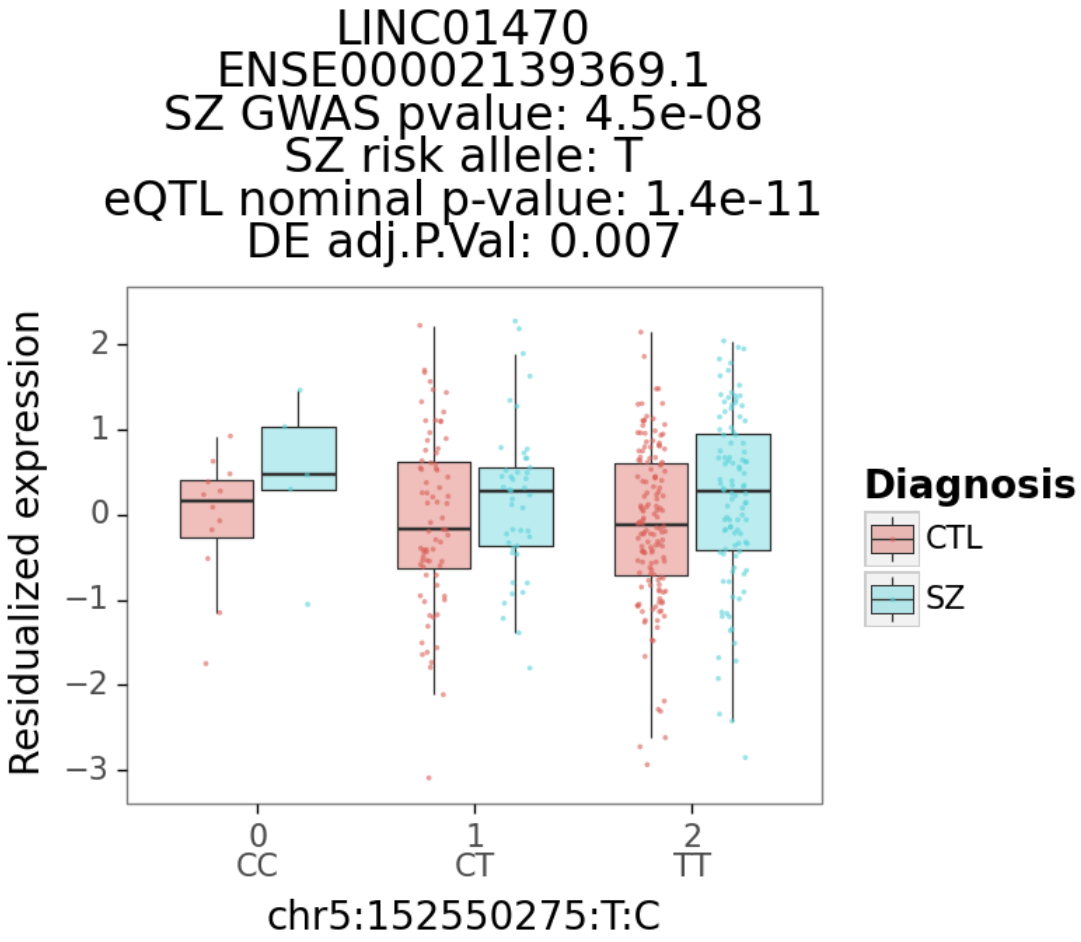


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