7 Hydroxylated Hif-1

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1 Table of Contents

In this notebook, I will identify genes that do not conform to the canonical epistasis relationships expected for the hypoxia pathway in *C. elegans*.

```
In [1]: # important stuff:
        import os
        import pandas as pd
        import numpy as np

        # TEA and morgan
        import genpy
        import gvars
        import morgan as morgan
        import tissue_enrichment_analysis as tea

        # Graphics
        import matplotlib as mpl
        import matplotlib.ticker as plticker
        import matplotlib.pyplot as plt
        import seaborn as sns
        import matplotlib.patheffects as path_effects
        from matplotlib import rc
        # rc('text', usetex=True)
        rc('text', usetex=True)
        rc('text.latex', preamble=r'\usepackage{cmbright}')
        rc('font', **{'family': 'sans-serif', 'sans-serif': ['Helvetica']})

        # Magic function to make matplotlib inline;
        %matplotlib inline

        # This enables SVG graphics inline.
        # There is a bug, so uncomment if it works.
```

```
%config InlineBackend.figure_formats = {'png', 'retina'}

# JB's favorite Seaborn settings for notebooks
rc = {'lines.linewidth': 2,
      'axes.labelsize': 18,
      'axes.titlesize': 18,
      'axes.facecolor': 'DFDFE5'}
sns.set_context('notebook', rc=rc)
sns.set_style("dark")

ft = 35 #title fontsize

mpl.rcParams['xtick.labelsize'] = 18
mpl.rcParams['ytick.labelsize'] = 18
mpl.rcParams['legend.fontsize'] = 14

genvar = gvars.genvars()
q=0.1

tidy_data = pd.read_csv('../output/temp_files/DE_genes.csv')
tidy_data.sort_values('target_id', inplace=True)
tidy_data.dropna(subset=['ens_gene'], inplace=True)
tidy_data = tidy_data[tidy_data.genotype != 'fog-2']
tidy_data['fancy genotype'] = tidy_data.code.map(genvar.fancy_mapping)
```

2 Genes that display non-canonical epistasis:

To identify genes that display non-canonical epistasis, I will fuse some columns to the dataframe containing the *rhy-1* transcriptome. Using these columns, we will find genes that have inverse expression changes between *vhl-1(lf)* mutants and *egl-9(lf)* or *rhy-1(lf)* mutants.

```
In [2]: # Specify the genotypes to refer to:
        single_mutants = ['b', 'c', 'd', 'e', 'g']

        # Specify which letters are double mutants and their genotype
        double_mutants = {'a' : 'bd', 'f':'bc'}

        # initialize the morgan.hunt object:
        thomas = morgan.hunt('target_id', 'b', 'tpm', 'qval')
        # input the genmap file:
        thomas.add_genmap('../input/library_genotype_mapping.txt', comment='#')
        # add the names of the single mutants
        thomas.add_single_mutant(single_mutants)
        # add the names of the double mutants
        thomas.add_double_mutants(['a', 'f'], ['bd', 'bc'])
        # set the q-value threshold for significance to its default value, 0.1
        thomas.set_qval()
        # Add the tpm files:
        kallisto_loc = '../input/kallisto_all/'
        sleuth_loc = '../sleuth/kallisto/'
        thomas.add_tpm(kallisto_loc, '/kallisto/abundance.tsv', '')
        # load all the beta dataframes:
        for file in os.listdir("../sleuth/kallisto"):
            if file[:4] == 'beta':
                letter = file[-5:-4].lower()
                thomas.add_beta(sleuth_loc + file, letter)
                thomas.beta[letter].sort_values('target_id', inplace=True)
                thomas.beta[letter].reset_index(inplace=True)
                thomas.filter_data()

        # place all
        df1 = thomas.beta['e'].copy()
        df2 = thomas.beta['b']
        df3 = thomas.beta['d']

        df1['b_b'] = df2.b
        df1['b_d'] = df3.b

        df1['q_b'] = df2.qval
        df1['q_d'] = df3.qval
```

```
In [3]: # use least strict conditions:
        lowestrhy = (df1.b*df1.b_d < 0)     # egl anti vhl

        lowestsigrhy = ((df1.qval < q) &    # egl sig
                        (df1.q_d < q))      # vhl sig


        lowestegl = (df1.b_b*df1.b_d < 0)   # egl anti vhl


        lowestsigegl = ((df1.q_b < q) &     # egl sig
                        (df1.q_d < q))      # vhl sig
```

Now that we have coded up the conditions, let's see what we get!

```
In [4]: df1.sort_values('qval', ascending=True)

        hifoh = df1[
                (lowestegl & lowestsigegl) |
                (lowestrhy & lowestsigrhy)].target_id.unique()

        print('{0} candidates found for HIF-1-OH regulation'.format(len(hifoh)))
        df1[(lowestegl & lowestsigegl) |
            (lowestrhy & lowestsigrhy)].to_csv('../output/temp_files/hifoh_candidates.csv', in
56 candidates found for HIF-1-OH regulation
```

```
In [5]: hypoxia = pd.read_csv('../output/temp_files/hypoxia_response.csv')
```

```
In [6]: len(hypoxia[hypoxia.target_id.isin(hifoh)].ens_gene.unique())
```

```
Out[6]: 14
```

3 Plotting genes that display non-canonical changes:

So far, all I have done is find the genes that have different expression between *vhl-1* and *egl-9*. It would be very interesting if genes that have these different behaviors still conform to the same epistatic rules (*egl-9* = *egl-9;vhl-1* and *hif-1* = *egl-9 hif-1*). We can make a qPCR plot to see if that is the case:

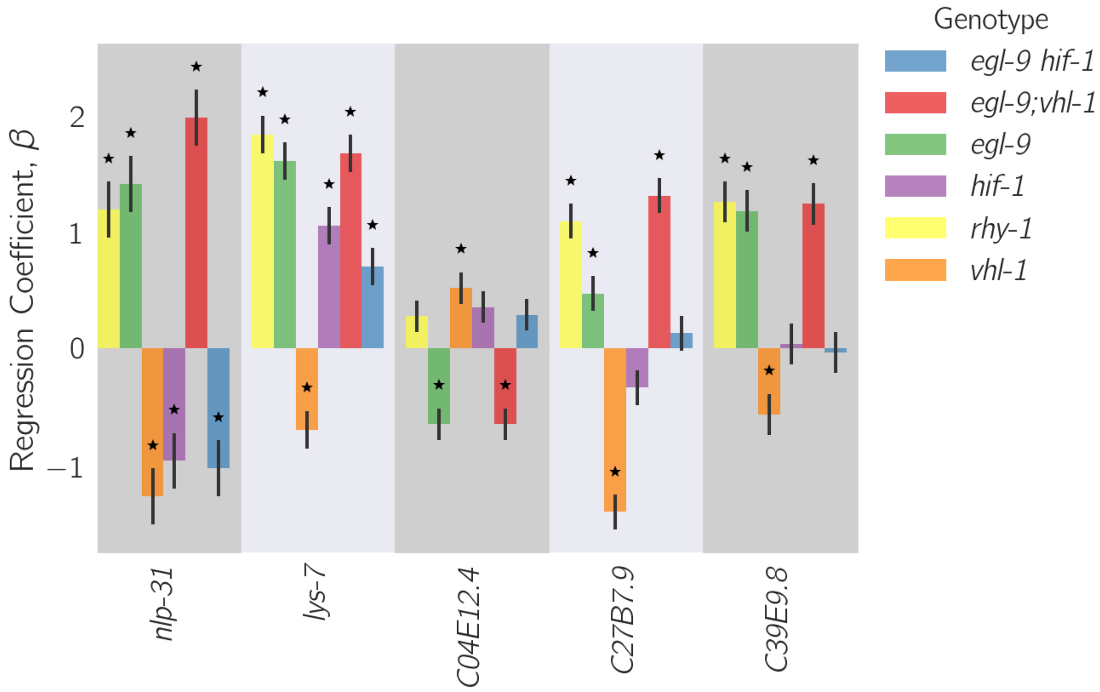
```
In [7]: tidy = tidy_data[tidy_data.target_id.isin(hifoh)].copy()

        x_sort = {}
        for i, xi in enumerate(tidy.target_id.unique()):
            x_sort[xi] = i + 1


        tidy['order'] = tidy.target_id.map(x_sort)
        tidy.sort_values('order', inplace=True)
        tidy.reset_index(inplace=True)
```

Initially, let's just look at the first five genes in our set:

```
In [8]: ind = tidy.target_id.isin(tidy.target_id.unique()[0:5])
        genpy.qPCR_plot(tidy[ind], genvar.plot_order, genvar.plot_color,
                        clustering='fancy genotype', plotting_group='target_id', rotation=90)
```



Wow! All of them obey the epistatic rules! This is cool.

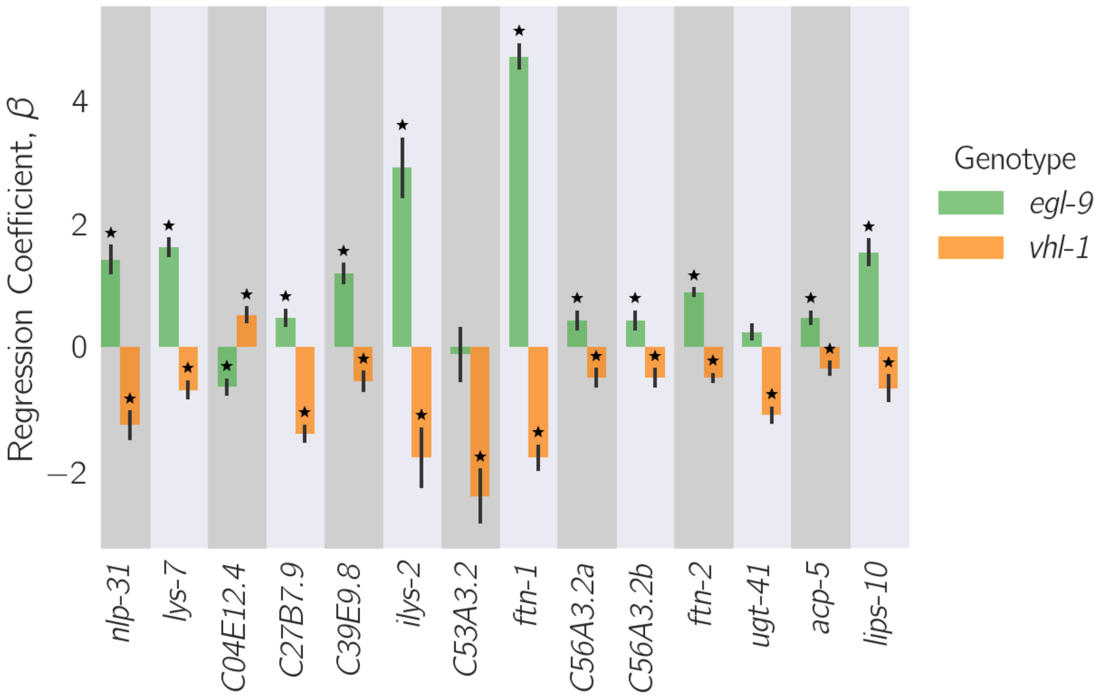
3.1 Figure 7A

Next, i will generate figure 7A and 7B in the paper.

```
In [10]: new_order = {r'\emph{egl-9}': 0,
                      r'\emph{vhl-1}': 1,
                     }

In [11]: genpy.qPCR_plot(tidy[(tidy.target_id.isin(hifoh[0:15])) & (tidy.code.isin(['b', 'd']))
                         new_order, genvar.plot_color, clustering='fancy genotype',
                         plotting_group='target_id', rotation=90)

         plt.savefig('../output/vhl1_noncanonical.svg')
```



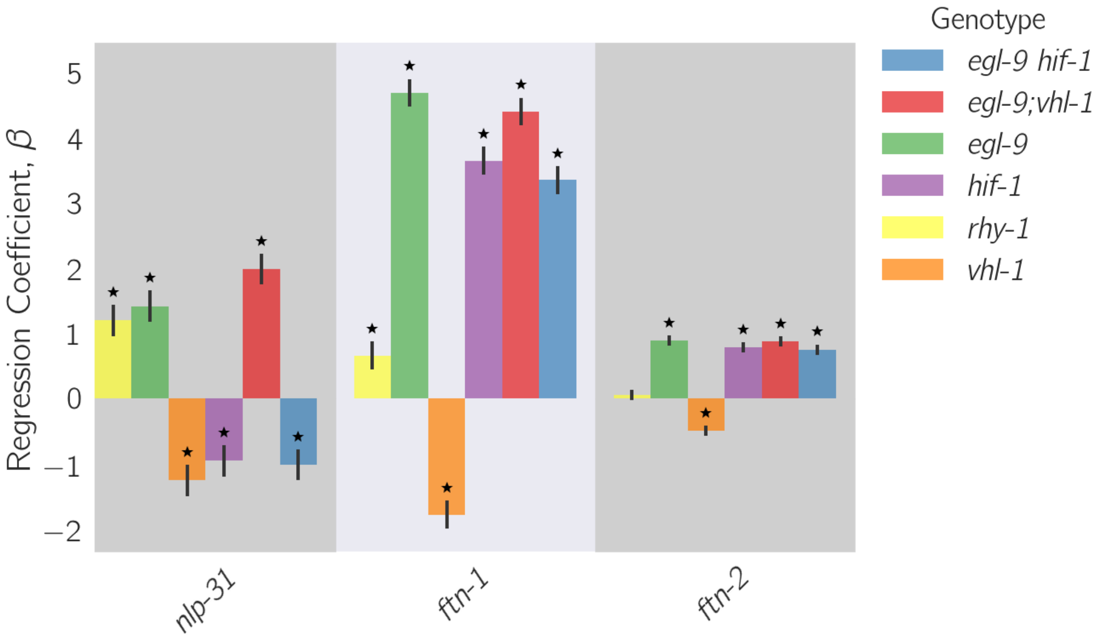
3.2 7B

```
In [12]: to_focus_on = ['nlp-31', 'ftn-1', 'ftn-2']

         gene_order = {'nlp-31': 1, 'ftn-1': 2, 'ftn-2': 3}

         temp = tidy[(tidy.ext_gene.isin(to_focus_on))].copy()
         del temp['order']
         temp['order'] = temp.ext_gene.map(gene_order)

         genpy.qPCR_plot(temp,
                         genvar.plot_order, genvar.plot_color, clustering='fancy genotype',
                         plotting_group='ext_gene')
         plt.savefig('../output/hif1oh_qPCR.svg', bbox_inches='tight')
```


Genotype
egl-9 hif-1
egl-9;vhl-1
egl-9
hif-1
rhy-1
vhl-1
Regression Coefficient, β
5
4
3
2
1
0
−1
−2
nlp-31
ftn-1
ftn-2

```
In [ ]:
```