4 Understanding the decoupled transcriptomes

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1 Table of Contents

In this notebook, I will identify gene targets that are specifically regulated by each *egl-9*, *vhl-1*, and *hif-1*. I define a specific regulatory node to mean the node that is the nearest regulatory node to these targets out of the subset of genes we have mutants for. As usual, we first load up all the libraries

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

        # morgan
        import tissue_enrichment_analysis as tea
        import epistasis as epi
        import genpy
        import gvars

        # Graphics
        import matplotlib as mpl
        import matplotlib.pyplot as plt
        import seaborn as sns
        from matplotlib import rc
        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.
        %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")

        mpl.rcParams['xtick.labelsize'] = 16
        mpl.rcParams['ytick.labelsize'] = 16
        mpl.rcParams['legend.fontsize'] = 14
```

```
In [2]: q = 0.1
        genvar = gvars.genvars()
        tissue_df = tea.fetch_dictionary()
        phenotype_df = pd.read_csv('../input/phenotype_ontology.csv')
        go_df = pd.read_csv('../input/go_dictionary.csv')
```

```
In [3]: 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['fancy genotype'] = tidy_data.code.map(genvar.fancy_mapping)
        tidy_data = tidy_data[tidy_data.genotype != 'fog-2']
        tidy_data.head()
```

```
Out[3]:               ens_gene ext_gene target_id         b      se_b      qval  \
        19676   WBGene00007064  2RSSE.1  2RSSE.1a  0.809959  0.586487  0.496563
        118056  WBGene00007064  2RSSE.1  2RSSE.1a  1.121038  0.586487  0.216276
        39352   WBGene00007064  2RSSE.1  2RSSE.1a  0.934036  0.586487  0.409735
        98380   WBGene00007064  2RSSE.1  2RSSE.1a  0.519789  0.586487  0.791051
        59028   WBGene00007064  2RSSE.1  2RSSE.1a  0.524134  0.586487  0.887525

                   genotype  sorter  code      fancy genotype
        19676         rhy-1       1     e        \emph{rhy-1}
        118056  egl-9;vhl-1       6     a  \emph{egl-9;vhl-1}
        39352         egl-9       2     b        \emph{egl-9}
        98380         hif-1       4     c        \emph{hif-1}
        59028   egl-9 hif-1       7     f  \emph{egl-9 hif-1}
```

2 Finding HIF-1 direct target candidates

We are interested in identifying gene targets of HIF-1. In order to do this, I will decouple my data into two parts: * a `positive` dataframe, which contains all genes with β values greater than 0 * a `negative` dataframe, which contains all genes with β values less than 0

I will also define a function called `collate`. This function takes in a list or a numpy array and returns a boolean indicator of what genes are in a specified dataframe. It's a lot shorter to define this function than it is to write the one-liner over and over again.

```
In [4]: def collate(x):
            """For a vector `x`, find what elements in x are contained in tidy_data.target_id.
            return tidy_data.target_id.isin(x)
```

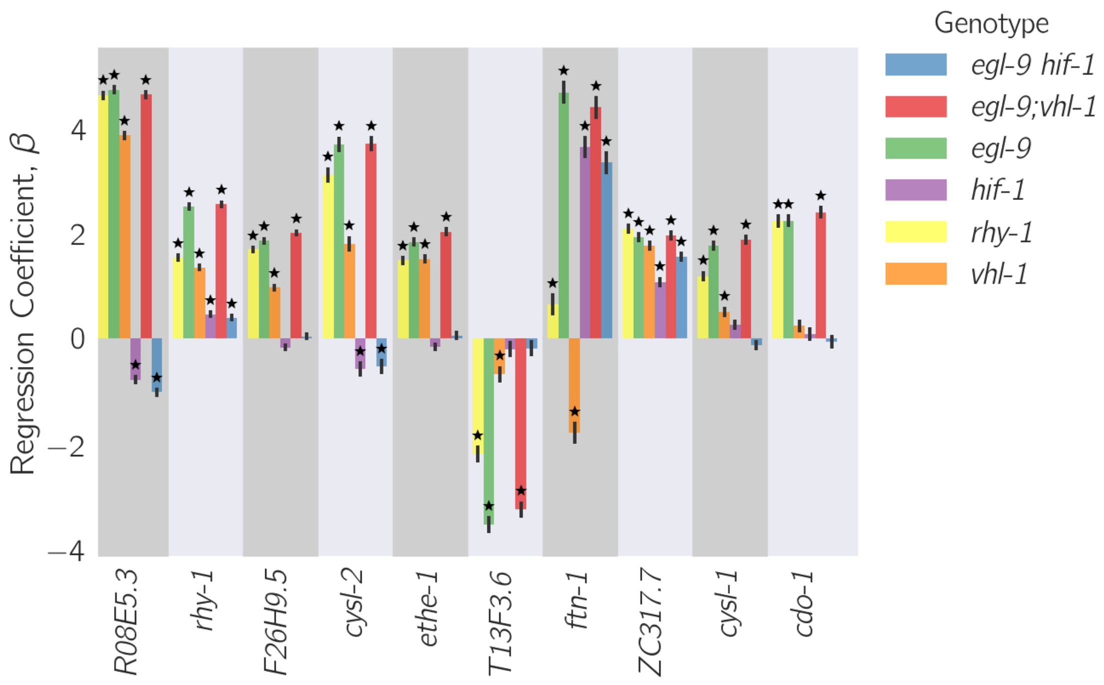
```
In [5]: hif_genes = pd.read_csv('../output/temp_files/hypoxia_response.csv')

        n = len(hif_genes[hif_genes.b > 0].ens_gene.unique())
        message = 'There are {0} unique genes that' +\
                  ' are candidates for HIF-1 direct binding'
        print(message.format(n))
```

```
There are 1173 unique genes that are candidates for HIF-1 direct binding
```

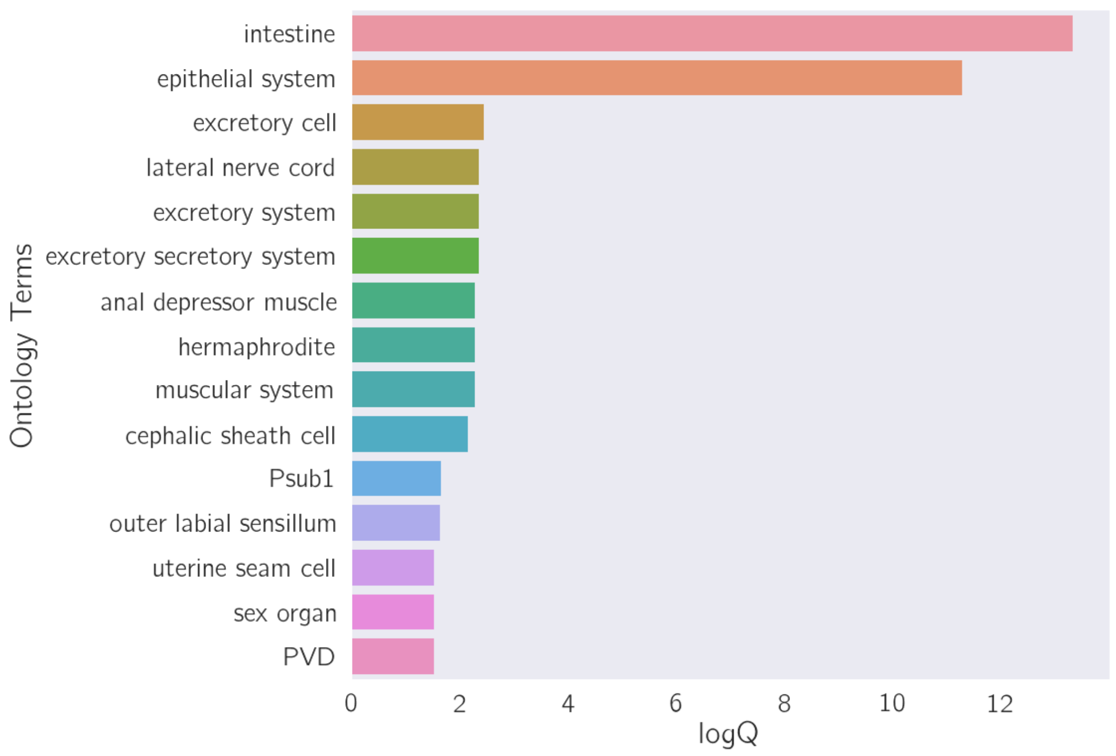
As a safety check, let's make a qPCR like plot to visualize our genes, and let's make sure they have the behavior we want:

```
In [6]: ids = hif_genes[hif_genes.b > 0].target_id
        hypoxia_direct_targets = tidy_data[tidy_data.target_id.isin(ids)]
```

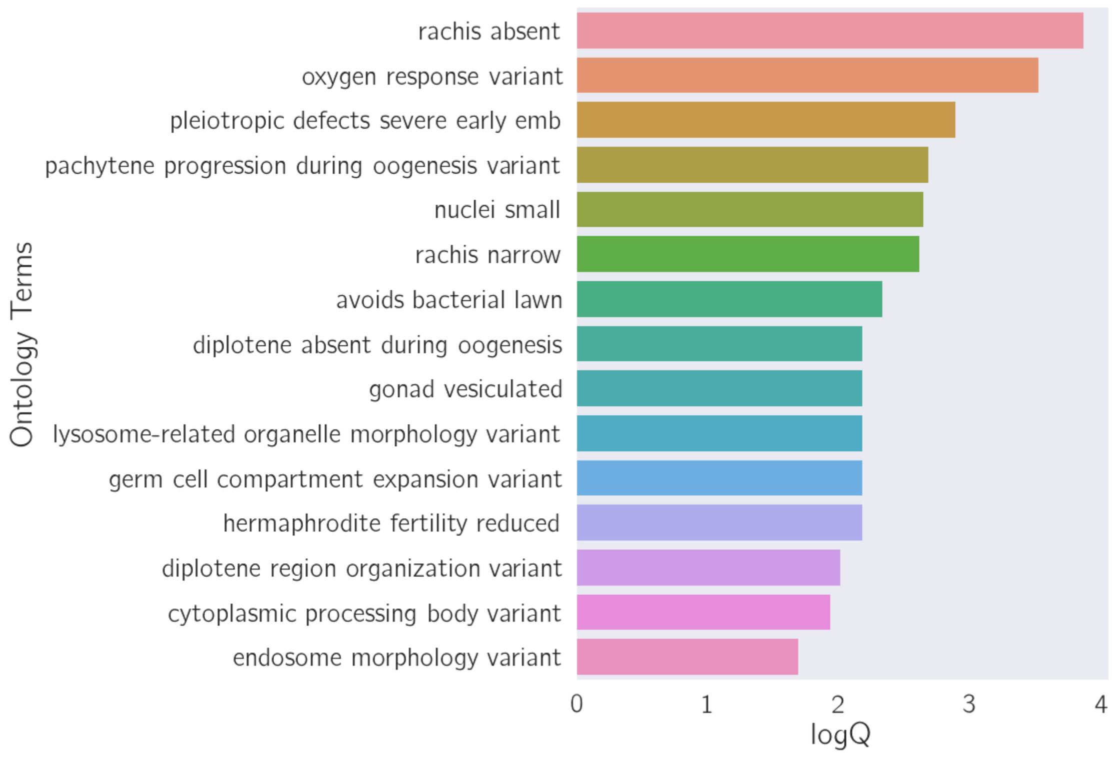
```
In [7]: names = hypoxia_direct_targets.sort_values('qval').target_id.unique()[0:10]

        name_sort = {}
        for i, name in enumerate(names):
            name_sort[name] = i+1

        plot_df = tidy_data[tidy_data.target_id.isin(names)].copy()
        plot_df['order'] = plot_df.target_id.map(name_sort)
        plot_df.sort_values('order', inplace=True)
        plot_df.reset_index(inplace=True)

        genpy.qPCR_plot(plot_df, genvar.plot_order, genvar.plot_color,
                        clustering='fancy genotype', plotting_group='target_id',
                        rotation=90)
```



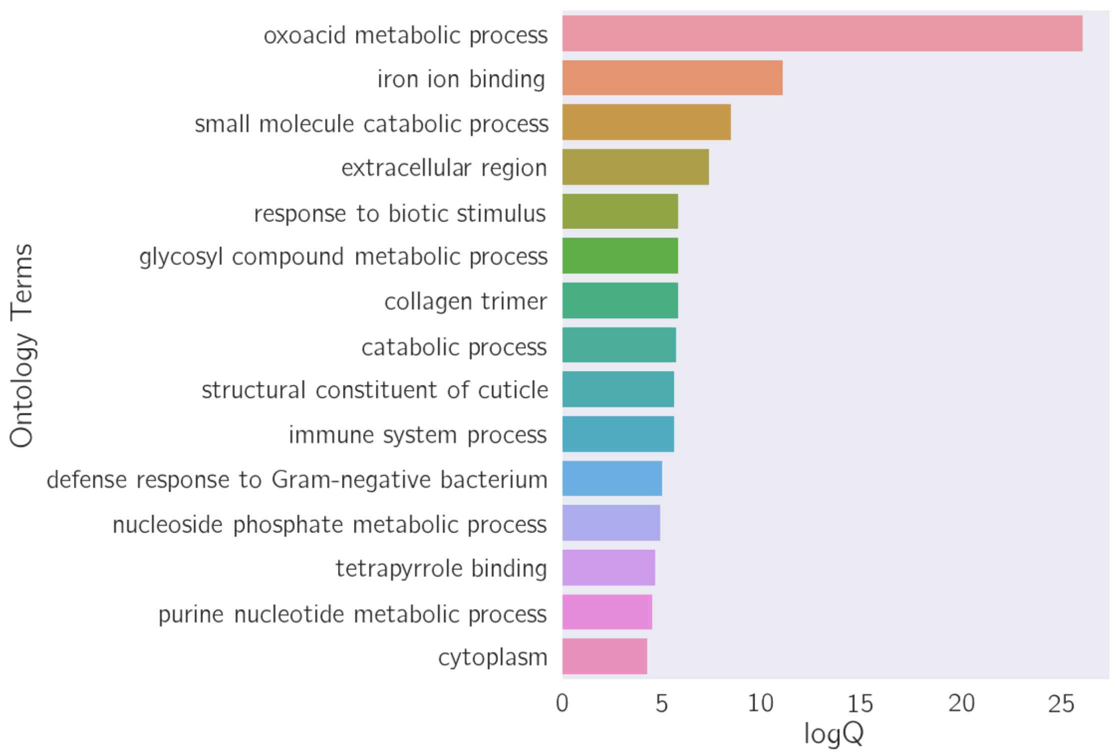
```
In [8]: res = tea.enrichment_analysis(hypoxia_direct_targets.ens_gene.unique(),
                                        tissue_df, show=False)
        res['logQ'] = -res['Q value'].apply(np.log10)
        tea.plot_enrichment_results(res, analysis='tissue', y='logQ')

Out[8]: <matplotlib.axes._subplots.AxesSubplot at 0x114b14400>
```



```
In [9]: res = tea.enrichment_analysis(hypoxia_direct_targets.ens_gene.unique(),
                                      phenotype_df, show=False)
        res['logQ'] = -res['Q value'].apply(np.log10)
        tea.plot_enrichment_results(res, analysis='phenotype', y='logQ')

Out[9]: <matplotlib.axes._subplots.AxesSubplot at 0x1149fb748>
```



```
In [10]: res = tea.enrichment_analysis(hypoxia_direct_targets.ens_gene.unique(),
                                       go_df, show=False)
         res['logQ'] = -res['Q value'].apply(np.log10)
         tea.plot_enrichment_results(res, analysis='go', y='logQ')

Out[10]: <matplotlib.axes._subplots.AxesSubplot at 0x1116cc4a8>
```



3 *vhl-1* dependent, *hif-1*-independent, genes

We can gate our settings to observe only *vhl-1*-dependent genes, by selecting only those genes that were present in the *vhl-1* and *egl-9;vhl-1* genotypes.

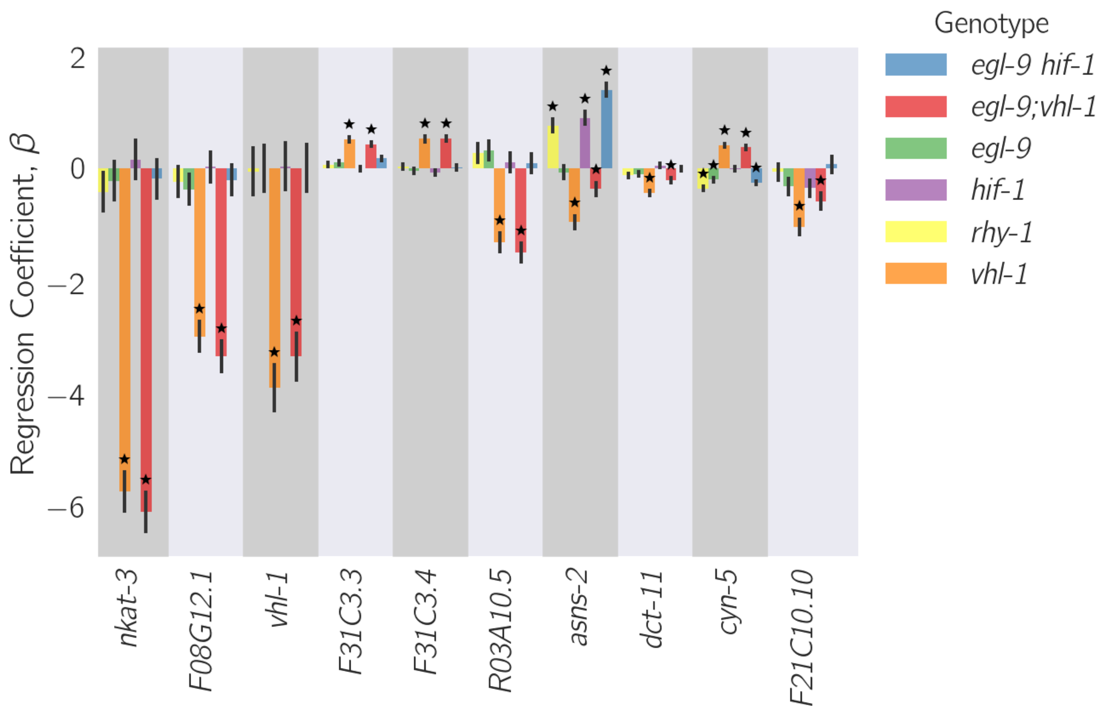
```
In [11]: positive = tidy_data[(tidy_data.qval < q) & (tidy_data.b > 0)]
         negative = tidy_data[(tidy_data.qval < q) & (tidy_data.b < 0)]

         # find the genes that overlap between vhl1 and egl-9vhl-1 and change in same directio
         vhl_pos = epi.find_overlap(['d', 'a'], positive)
         vhl_neg = epi.find_overlap(['d', 'a'], negative)
         vhl = list(set(vhl_pos + vhl_neg))

         # find genes that change in the same direction in vhl(-) and vhl(+ datasets)
         same_vhl = []
         for genotype in ['b', 'e', 'f', 'c']:
             same_vhl += epi.find_overlap(['d', 'a', genotype], positive)
             same_vhl += epi.find_overlap(['d', 'a', genotype], negative)

         # put it all together:
         ind = (collate(vhl)) & (~collate(same_vhl))
         vhl_regulated = tidy_data[ind & (tidy_data.code == 'd')]
```

```
        n = len(vhl_regulated.ens_gene.unique())
        message = 'There are {0} genes that appear to be ' + \
                  'regulated in a hif-1-independent, vhl-1-dependent manner.'
        print(message.format(n))
```

```
There are 72 genes that appear to be regulated in a hif-1-independent, vhl-1-dependent manner.
```

3.1 Plot *vhl-1*-dependent, *hif-1*-independent genes

```
In [12]: # begin plotting
         names = vhl_regulated.sort_values('qval').target_id.unique()[0:10]
         name_sort = {}
         for i, name in enumerate(names):
             name_sort[name] = i+1

         plot_df = tidy_data[tidy_data.target_id.isin(names)].copy()
         plot_df['order'] = plot_df.target_id.map(name_sort)
         plot_df.sort_values('order', inplace=True)
         plot_df.reset_index(inplace=True)

         genpy.qPCR_plot(plot_df, genvar.plot_order, genvar.plot_color,
                         clustering='fancy genotype', plotting_group='target_id',
                         rotation=90)

         # save to file
         cols = ['ext_gene', 'ens_gene', 'target_id', 'b', 'qval']
         vhl_regulated[cols].to_csv('../output/temp_files/vhl_1_regulated_genes.csv')
```



No enrichment was observed for these genes.