

On Cytoscape (<https://cytoscape.org/>), the stringApp, Largest Subnetwork app and Legend Creator were installed. The STRING database was queried for protein interactions between the list of genes copied from the filtered female DESeq2 CSV file along with *SIAH2*, which was the gene knocked out in the study. The species was set to *Mus musculus*. There were some genes separated from the main network, so the nodes in the largest subnetwork were selected and a new network built from them. The style was changed to default and the node shape to ellipse, with locked node width and height.

The rest of the data in the CSV was imported as node data columns using the STRING query term as the key column. Then the log2FoldChange values were used to set the node fill colour with continuous mapping. The colour went from white to blue for negative log2FoldChange, indicating downregulation, and white to red for positive log2FoldChange, indicating upregulation. The default node colour was set to green so *SIAH2* could be seen clearly.

A network analysis was carried out and results shown In Figure 1. The degree, which shows a power law distribution indicating a scale-free network, was then used for continuous mapping of node size from 35 to 80. A legend was also added. The final network is shown in Figure 2.

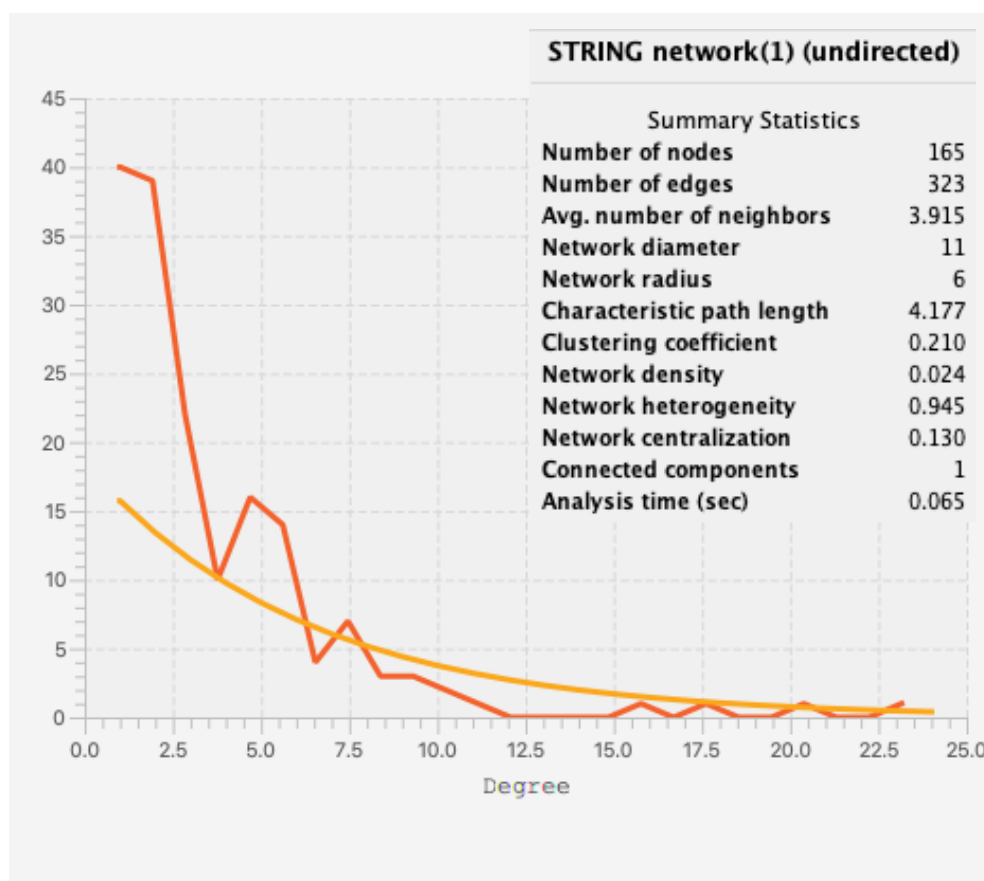


Figure 1: Node degree distribution graph and summary statistics for network

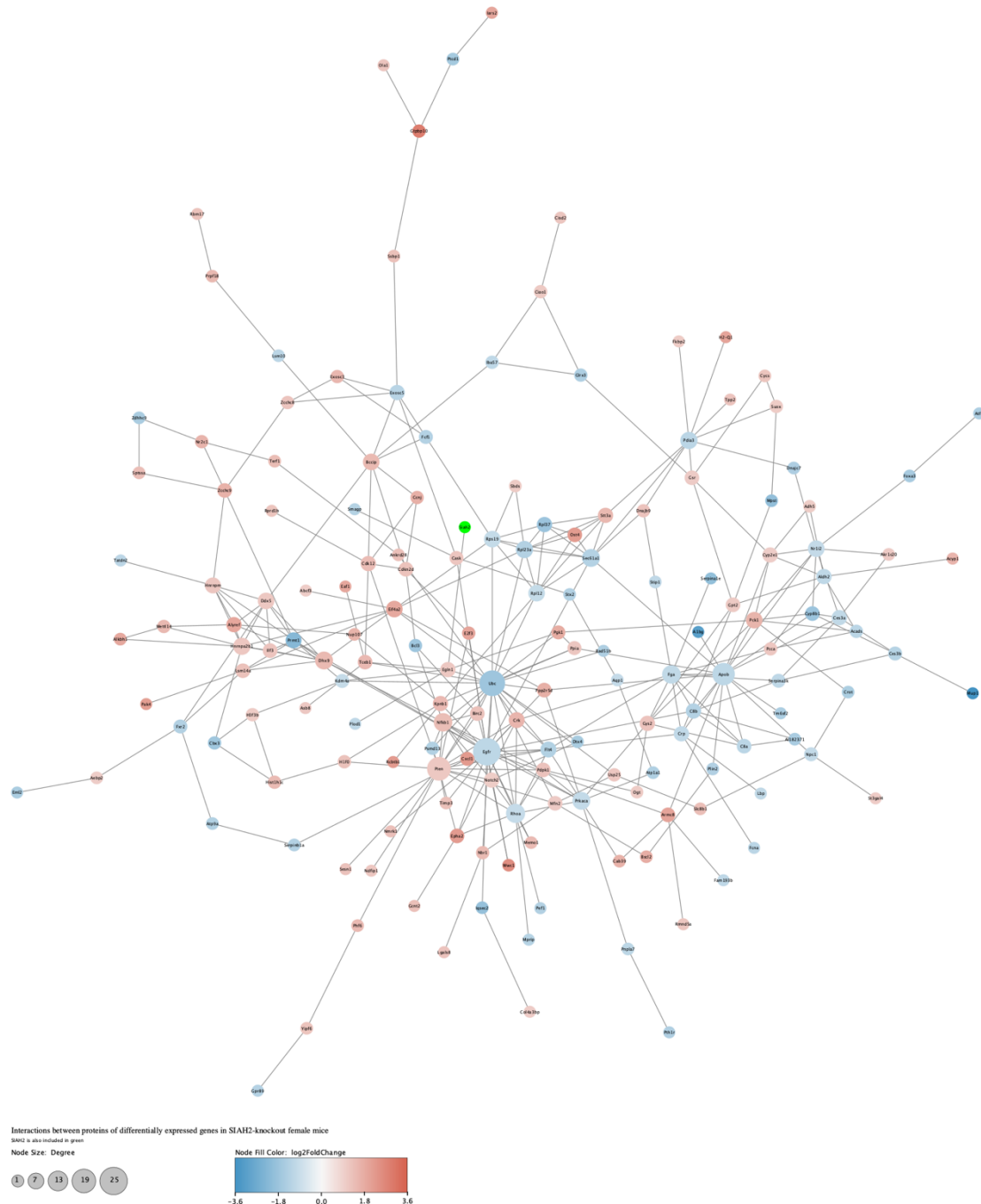


Figure 2: Cytoscape network of interactions between proteins of differentially expressed genes in *SIAH2*-knockout female mice

In order to clearly see the effect of perturbations on the network, new networks were built from the first, second and third neighbours of *SIAH2* and first neighbours of *UBC*, a hub gene in the network. Default style was used as before. A force-directed layout was applied to both networks, as shown in Figures 3 and 4. *SIAH2* and *UBC* were then cut from their respective networks and force-directed layouts were applied again to see the effect, as shown in Figures 5 and 6.

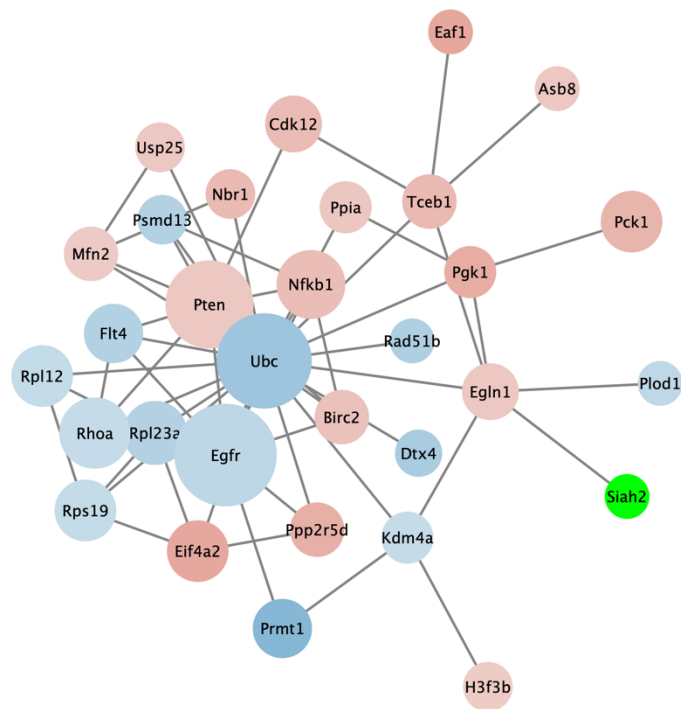


Figure 3: Force-directed layout network of *SIAH2* first, second and third neighbours

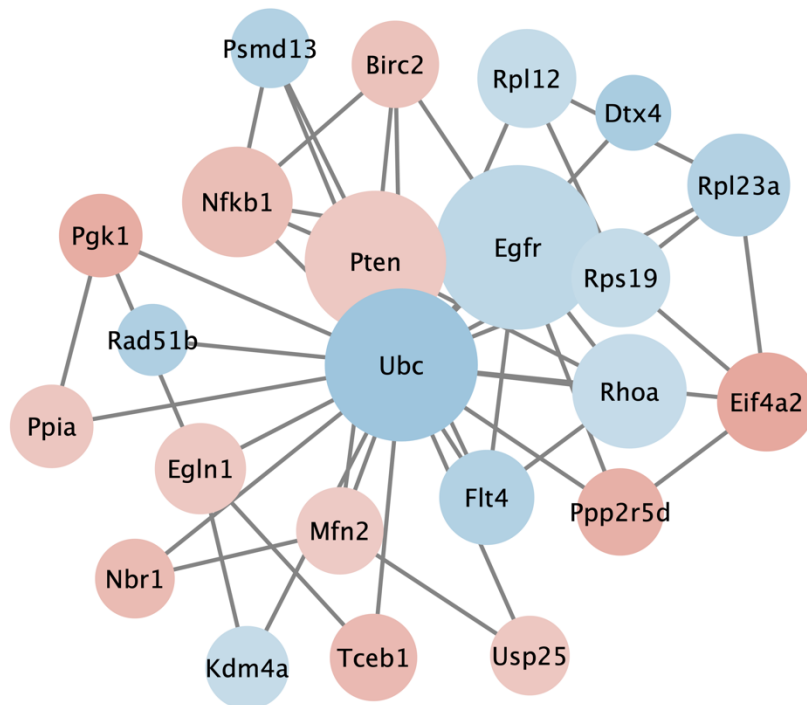


Figure 4: Force-directed layout network of *UBC* first neighbours

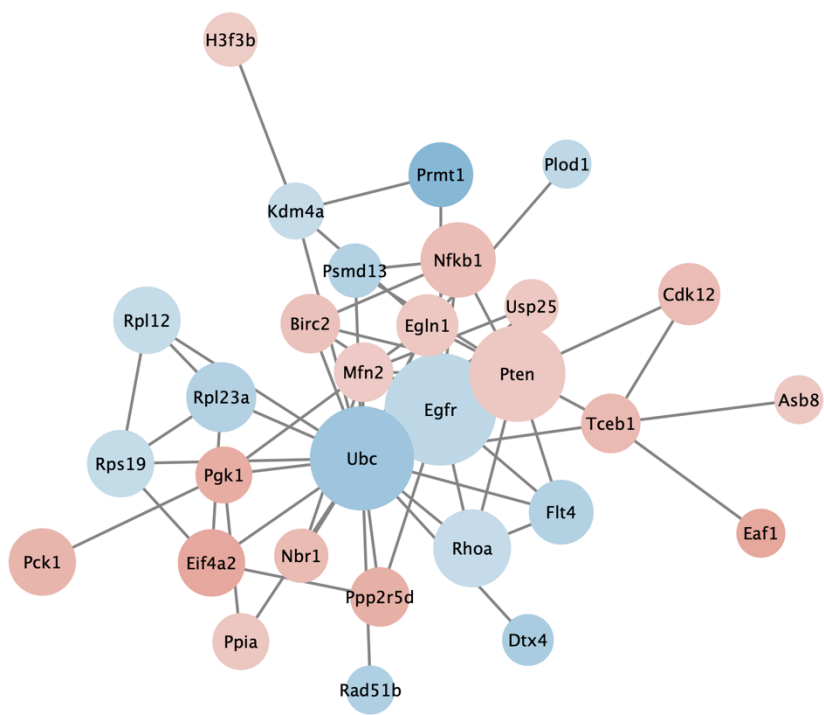


Figure 5: Force-directed layout network of *SIAH2* first, second and third neighbours with *SIAH2* removed

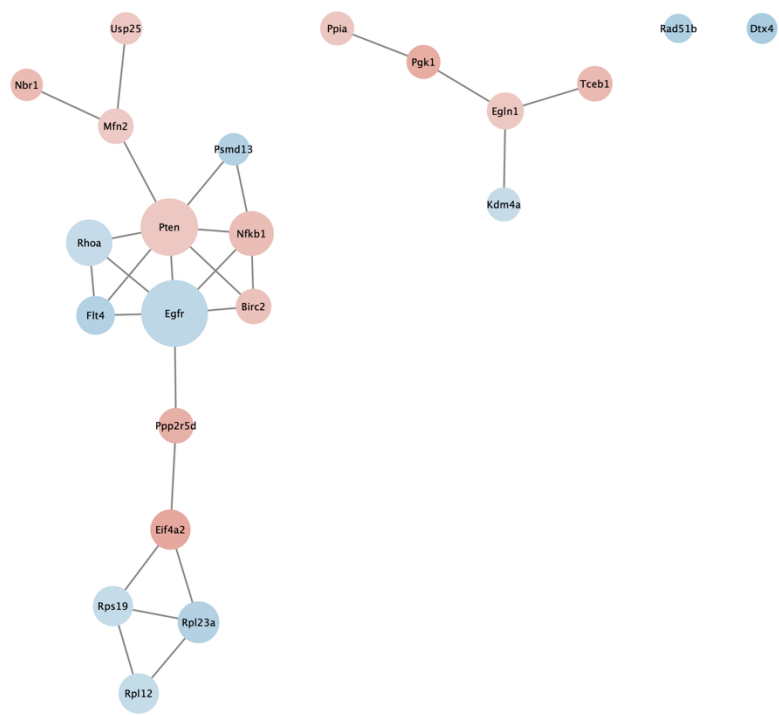


Figure 6: Force-directed layout network of *UBC* first neighbours with *UBC* removed

As expected given their respective degrees, *SIAH2* removal had much less of an effect than *UBC* removal, which resulted in separation of the network and indicates an area of fragility. However, the fact that all the genes in the network were differentially expressed in female mice with *SIAH2* knocked out suggests there may be more interactions to be found. Perhaps most interaction data is based on male models, while this mechanism is specific to females. Alternatively, maybe *SIAH2* is involved in regulating one of its neighbour hub genes, such as *UBC*, which is downregulated in knockout mice, and that is how it impacts all the other genes.

PNG files of all the images can be found at <https://github.com/bethsampher/systems-biology-coursework>, along with the network exported in XGMML format and the saved Cytoscape session.