**Supplementary 5: Statistical network analysis**

**Network visualization**

The topology of the mouse network was visualized using Force-Directed Kamada-Kawai visualization algorithm\(^1\) and GEM.\(^2\) Kamada-Kawai model generates a balanced metabolic network graph with uniform distribution and symmetry; every pair of nodes is considered to be connected by an imaginary spring, each of which has its own spring constant and ideal length determined by the geodesic distance of the pair. In order to decrease total imbalance of layout, it attempts to lower the summation of the spring potential energy of every two node pairs:

\[
E = \sum_{i=1}^{n} \sum_{j=i+1}^{n} \frac{1}{2} k_{ij} ((\text{current distance of } (i,j) - \text{ideal distance of } (i,j))^2
\]

An iterative step is involved to reduce the imbalance as well as to improve the convergence into the final configuration that results in an acceptable network structure.

GEM is another force-directed model, but it is known as its faster convergence with various concept of impulse, oscillation and rotation and attempts to avoid local minima by employing simulated annealing. In this paper, GEM is used for drawing some *Mus Musculus* metabolic networks, since the GEM visualization output among various force-directed algorithms is most aesthetically appealing.

**Statistical network analysis**

The network was statistically analyzed using degree and betweenness centrality techniques for the identification of highly linked and bridging metabolites in the network. For calculating the centrality of each metabolite, the network was modified in such a way that it considers only the
interactions/links among the nodes i.e., links between the reactants/products without considering information on reaction names, subsystems and pathways.

**Degree centrality**: A degree centrality of a node is the number of its incident links divided by a normalization factor:

$$\text{Degree centrality of a node (v)} = \frac{\text{Number of the incident links of the node (v)}}{\text{Number of nodes} - 1}$$

Degree centrality measure is typically used to identify hub metabolites with a high degree of links.

**Betweenness centrality**: A betweenness centrality of a node is the number of shortest paths over (v) between every pair of nodes divided by a normalization factor:

$$\text{Betweenness centrality of a node (v)} = \frac{\text{Number of shortest paths over the node (v)}}{\frac{(n-1)(n-2)}{2}}$$

The betweenness centrality can be used to represent the degree of bridging roles in a network.

**References**