Multi-omic network-based interrogation of rat liver metabolism following gastric bypass surgery featuring SWATH proteomics

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Morbidly obese patients often elect for Roux-en-Y gastric bypass (RYGB), a form of bariatric surgery that triggers a remarkable 30% reduction in excess body weight and reversal of insulin resistance for those who are type II diabetic. A more complete understanding of the underlying molecular mechanisms that drive the complex metabolic reprogramming post-RYGB could lead to innovative non-invasive therapeutics that mimic the beneficial effects of the surgery, namely weight loss, achievement of glycemic control, or reversal of non-alcoholic steatohepatitis (NASH). To facilitate these discoveries, we hereby demonstrate the first multi-omic interrogation of a rodent RYGB model to reveal tissue-specific pathway modules implicated in the control of body weight regulation and energy homeostasis. In this study, we focus on and evaluate liver metabolism three months following RYGB in rats using both SWATH proteomics, a burgeoning label free approach using high resolution mass spectrometry to quantify protein levels in biological samples, as well as MRM metabolomics. The SWATH analysis enabled the quantification of 1378 proteins in liver tissue extracts, of which we report the significant down-regulation of *Thrsp* and *Acot13* in RYGB as putative targets of lipid metabolism for weight loss. Furthermore, we develop a computational graph-based metabolic network module detection algorithm for the discovery of non-canonical pathways, or sub-networks, enriched with significantly elevated or depleted metabolites and proteins in RYGB-treated rat livers. The analysis revealed a network connection between the depleted protein *Baat* and the depleted metabolite taurine, corroborating the clinical observation that taurine-conjugated bile acid levels are perturbed post-RYGB.

Keywords: Obesity; Roux-en-Y; Gastric Bypass; SWATH Proteomics; Metabolomics.

INNOVATION

The innovation of this work is two-fold: First is the application of SWATH proteomics, a state-of-the-art label free work flow using high resolution LC/MS-MS technology, to obtain physiological insight into the liver metabolic remodeling that occurs following gastric bypass, thereby elucidating the molecular mechanisms behind the weight loss and increased insulin sensitivity. Second is a novel computational approach to integrate metabolomics and proteomics data using a metabolic network-based algorithm that can efficiently capture and visualize differentially expressed metabolic enzyme levels along with the relative concentrations of metabolites, whose production and consumption are catalyzed by those enzymes. Hence, we present a generally applicable workflow of multi-omics analysis that can be applied to any *in vivo* or *in vitro* experimental system. We demonstrate the utilization of this innovation in the context of understanding the effects of rodent gastric bypass surgery, which can facilitate the discovery of novel therapeutic targets for metabolic diseases.

INTRODUCTION

Obesity, broadly characterized by excess accumulation of body fat, affects approximately 35% of the adult US population¹ and is usually associated with several co-morbidities that define the metabolic syndrome, including type II diabetes, hypertension, and non-alcoholic fatty liver disease (NAFLD). Unfortunately, FDA-approved obesity therapeutics have historically been marred by safety concerns and newly approved drugs offer very modest efficacies. For example, Lorcaserin (Arena Pharmaceuticals/Eisai), a serotonin receptor agonist modulating appetite, was recently approved for obesity in 2012, but clinical trials demonstrated only a 3–4% placebo-adjusted sustained reduction in body weight². In contrast, gastric bypass surgery remains a far more effective treatment option, where patients opting for the procedure to reconfigure gastrointestinal tract anatomy and enable food to bypass the stomach and parts of the intestine, sustain an average of 30% reduction in body weight post-operatively. Since Roux-en-Y gastric bypass (RYGB, the most common form of the surgery) stimulates the secretion of various hormones³ and results in

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the reprogramming of metabolically active tissues, the rapid weight loss involves a more complex mechanism than can be solely explained by the reduction in food intake by patients post-surgery. Unfortunately, RYGB surgery, while therapeutically very effective, is highly invasive and presents risk of further complication. As such, understanding the molecular mechanisms of action that trigger the metabolic shifts following RYGB offers an avenue to discover novel non-invasive therapies against symptoms of the metabolic syndrome.

In this light, several clinical studies have been conducted on bariatric surgery patients to collect tissue, urine, and serum samples and experimentally quantify various biomolecules post-surgery using proteomics^{4,5} and metabolomics^{6,7} approaches. However, access to intestinal, liver, and visceral fat tissue post-surgery is highly invasive and therefore information on the metabolic dynamics in these specific organs has largely been elusive based on human clinical studies. For further investigation of various metabolically active tissues following the surgery, rodent models for RYGB that mimic the weight loss and resolution of glycemic control in humans have offered a controlled experimental platform to capture the system-wide impacts of the surgery^{8–10}. These animal models have offered insight into the effects of various gut-derived hormones such as Glp1, the impact of the gut-microbiota in modulating weight loss¹¹, and the altered metabolism of bile acids, some of which have been implicated as agonists of FXR, a therapeutic target for NAFLD¹². In particular, Saeidi and coworkers recently demonstrated the first mechanism of action for the rapid improvement in glycemic control following RYGB in rats, based on evidence that glucose uptake in intestinal tissue was highly up-regulated¹⁰.

The clinical observation that RYGB elicits a perturbation to bile acid metabolism and secretion suggests that a similar metabolic reprogramming of hepatocytes occurs following the surgery and further interrogation of the liver could help unravel the underlying mechanism of RYGB's long term effects. In this study, we exploit SWATH¹³ (Sequential Window Acquisition of all Theoretical fragments), a relatively new label-free mass spectrometry technique for targeted proteomics, for the global profiling of rat liver tissue metabolism three months following Sham and RYGB treatment. SWATH proteomics is increasingly being utilized for translational medicine applications^{14–16} since it offers an impressive coverage of the target proteome as recently demonstrated by Rosenberger and coworkers, who developed a SWATH-based assay capable of identifying 10,316 proteins from human samples¹⁷. Since a subset of the proteins identified using the technique are metabolic enzymes catalyzing the production and consumption of small molecule metabolites, we also complemented the SWATH data with targeted metabolomics analysis on the same liver tissue extracts using multiple reaction monitoring (MRM)¹⁸. As with all omics analysis, interpretation of large multi-dimensional data to provide insightful biological insight remains challenging. We therefore also develop and present a novel computational network-based integration of proteomics and metabolomics data to identify reaction modules involved in metabolism that are enriched with statistically significant proteins and metabolites (between Sham and RYGB treated groups) using a local neighborhood search algorithm.

In summary, we report the quantification of 1378 proteins in liver extracts from rats subject to either Sham or RYGB treatment, of which 82 exhibited a statistically significant difference in expression between the experimental groups. A cursory analysis of the data revealed a perturbation to proteins involved in iron metabolism and a couple of proteins known to regulate lipid metabolism (Thrsp, Acot13), both of which have been previously validated by others as putative obesity targets based on body weight reduction in knockout mice. Moreover, a more rigorous analysis of the multi-omic integration using the network modularity approach revealed complex perturbations to modules otherwise classified as reactions from “one carbon folate metabolism” and “bile acid synthesis”, the latter highlighting taurine conjugation of bile acids, which are known to correlate with obesity metrics in the clinic.

Our experimental and computational workflow should serve as a novel template for future studies in translational medicine featuring multi-omic integration to evaluate complex metabolic processes and discover novel therapeutic targets.

RYGB MULTI-OMIC INTEGRATION: METHODOLOGY AND RESULTS

Physiological outcomes of RYGB and -omic workflow

The anatomical reconstruction of RYGB in rats is highlighted in **Fig. 1a–c** and the surgical methods for both RYGB and sham surgery were similar to those previously described in detail⁹. Briefly, diet-induced obese (DIO) male Sprague Dawley rats (Charles River Laboratories) were fasted overnight and anesthetized using 2–4% isoflurane. In the RYGB surgery, a small gastric pouch was created using a laparoscopic stapler (Ethicon, Cincinnati, OH). The jejunum was transected and anastomosed to the gastric pouch to create the Roux Limb (RL) and the proximal transected end of the duodenum was anastomosed to the side of the distal jejunum to create the biliopancreatic limb (BP) restore intestinal tract continuity. The sham operation, which omits the step of the surgical procedure regarded as the therapeutic necessity, was performed on age and weight-matched rats. Briefly, this involved a laparotomy, where the ligament of Treitz is identified, and the same segment of the intestine was transected as in the real RYGB surgery. However, instead of constructing biliopancreatic and roux limbs, the two transected segments were simply anastomosed back together. A hole was constructed in the forestomach where the roux limb would be attached and then repaired, after which the laparotomy was closed^{10,19}.

The RYGB-treated animals undergo a statistically significant ~25% weight loss (**Fig. 1d**) that was sustained for the entire duration of this study (3 months). In addition, histological samples demonstrate that while the liver in the sham animal contain large number of lipid droplets, which is a hallmark of hepatic steatosis, the condition is completely resolved in the RYGB animals (**Fig. 1e,f**). In this study, we present a generally applicable experimental and computational workflow to interrogate tissue metabolism of an *in vivo* system, which as highlighted in **Fig. 1g**, involves harvesting liver tissue biopsies and employing LC/MS-MS analytical platforms for targeted proteomics and metabolomics data.

Coverage of SWATH proteomics

The SWATH proteomics analysis is based on AB Sciex Tripletof LC/MS-MS technology and is described in more detail in **Supplementary Methods**. The bioinformatics workflow used to map the high resolution MS/MS spectra to known trypsin-digested peptides enabled the relative quantification of 1378 proteins in rat liver tissue extracts in at least one of the Sham or RYGB treated animals. To determine the coverage and utility of SWATH proteomics for rat liver metabolism, we compiled a list of rat liver proteins belonging to each KEGG pathway²⁰ that were detected by SWATH in at least one sample and computed the fractional coverage of each pathway by dividing the number of detected proteins by the total number of expressed genes in rat. For example, the pathway “Glyoxylate and dicarboxylate metabolism” yielded complete coverage, in that for all 28 rat genes catalogued in KEGG, the corresponding protein was quantified in rat liver using SWATH (**Table 1**). In contrast, out of the 1249 genes catalogued in “Olfactory transduction”, only two proteins were detected in liver (**Supplementary Table 5**), which is unsurprising considering the liver does not function in smell detection.

Effect of RYGB on rat liver proteome

Of the 1378 proteins that were detected and quantifiable in rat liver extracts using SWATH, 45 proteins are statistically significantly elevated in RYGB samples compared to Sham and 37 are depleted in RYGB (**Fig. 2a**), for a total of 82 Statistically Significant Proteins (SSPs) based on the Wilcoxon rank test (the ranksum function in Matlab[®]). The Wilcoxon rank test is a

Figure 1 RYGB in rats induces systemic and hepatic-specific metabolic improvements. (a,b) Schematic drawing and (c) intraoperative image showing the anatomical reconfiguration of the gastrointestinal tract following RYGB with the following annotations: ▲: common limb, ←: site of anastomoses between the biliopancreatic limb and common limb, ★: the biliopancreatic limb, ◆: the roux limb (d) At three months post-surgery, the RYGB-treated rats sustainably have lost ~25% of their pre-operative body weight. In contrast, the sham-treated rats continuously gained weight throughout the duration of the experiment. (e) Histological analysis of liver biopsies from both groups clearly demonstrate hepatocellular ballooning and steatosis in sham animals, which was completely resolved in RYGB-operated animals (f). (g) The multi-omic experimental workflow for the study.

non-parametric test to determine the likelihood that two samples are from the same distribution of equal medians, and is often used for *in vivo* experiments with small sample sizes where the residuals of the measurements are not normally distributed. The complete list of the calculated SSPs, along with a description of their function and relation to energy metabolism, or the metabolic syndrome is presented in **Supplementary Table 1**. Principal component analysis shows separation between the two treatment groups, with the separation driven by the second principal component (**Fig. 2b**). The absolute value of PC2's coefficients were sorted by magnitude and the

Table 1 Fractional coverage of KEGG pathways using SWATH proteomics in rat liver samples.

^A KEGG pathway	^B Rat genes	^C SWATH proteins	^D Coverage
1. Glyoxylate and dicarboxylate metabolism	29	29	1.00
2. Lysine biosynthesis	2	2	1.00
3. Citrate cycle (TCA cycle)	32	29	0.91
4. Propanoate metabolism	32	28	0.88
5. Valine, leucine, and isoleucine degradation	56	42	0.75
6. 2-oxocarboxylic acid metabolism	19	14	0.74
7. Fatty acid degradation	47	33	0.70
8. Primary bile acid biosynthesis	16	11	0.69
9. D-Glutamine and D-glutamate metabolism	3	2	0.67
10. Proteasome	47	31	0.66

top 14 proteins (1.0% of the measured 1378) that comprise PC2 and are also differentially expressed between treatment groups include Aldh9a1, Nme2, Tfrc, Scep1, Igkc, Fth1, Acat2, Ociad2, Ndufv2, Fabp7, Bhmt, Map1, Ephx, and Snrpd1. Notably, two of these proteins (Tfrc, and Fth1) are involved in iron acquisition and storage (**Fig. 2c**), which could explain the anemia patients experience post-RYGB. Furthermore, we performed Pathway Enrichment Analysis using the canonical classifications for each protein offered by KEGG, where the total number of statistically significant proteins (SSPs) classified under each pre-defined pathway was calculated. 100 pathways for *Rattus norvegicus* comprise at least one protein that was differentially expressed between Sham and RYGB, of which 47 comprise two or more proteins (**Supplementary Table 2**). This analysis provides a rudimentary assessment of which known metabolic and signaling pathways are affected by RYGB, but is subject to the inherent bias associated with pre-defining pathway boundaries.

Network analysis and metabolomics integration

For a more un-biased analysis of -omics data, we introduce a graph network-based algorithm to identify modules, or sub-networks spanning multiple canonical pathways, that are impacted by RYGB. For this analysis, the Recon 2.1 metabolic model, describing 7440 reactions in human metabolism, was downloaded from humanmetabolism.org²¹, and bipartite and reaction-centric graph networks were constructed as described in **Supplementary Methods**. Of the 1378 proteins detected in rat liver using SWATH, 1266 proteins had a corresponding gene entry catalogued in the KEGG database under the *Rattus norvegicus* genome and 575 possessed an enzyme commission number (EC) associated with the gene suggesting its involvement in metabolism. Of these, 346 proteins catalyze at least one of the 7440 reactions described in the Recon 2.1 network. Since a statistically significant protein (SSP) between Sham and RYGB can catalyze more than one network reaction in Recon 2.1 spanning multiple compartments, we distinguish SSPs from Statistically Significant Reactions (SSRs). For each measured protein that was a metabolic enzyme, the corresponding reactions in the Recon 2.1 metabolic network were identified based on EC numbers. For metabolomics data, collected using Multiple Reaction Monitoring (MRM) based on a protocol described by Yuan *et al.*¹⁸, 303 metabolites were quantified from the crushed rat liver samples. Comparing metabolite levels between Sham and RYGB, we computed the number of Statistically Significant Metabolites (SSMs, also determined using the Wilcoxon rank test with a *p*-value threshold of 0.05 and reported in **Supplementary Table 3**) in each KEGG pathway, a computation that is similar to the pathway enrichment tool provided by MetaboAnalyst²². We find that pyrimidine metabolism, purine metabolism,

Figure 2 (a) Heatmap of SWATH Proteomics data where the relative abundance of each protein is color-coded based on the z-score of the protein's normalized peak area. (b) Principal component analysis reveals separation between Sham and RYGB conditions (c) Selected statistically significant proteins (SSPs) highlighting perturbation to iron metabolism (Tfr, Fth1), and potential therapeutic liver targets for obesity (Thrsp, Acot13).

and glutathione metabolism were canonical pathways most enriched with SSMs, independent of proteomic information (**Supplementary Table 4**). Since the solvent-based metabolite extraction procedure on crushed tissue does not enable one to determine which compartment was responsible for the difference in metabolite level between Sham and RYGB, we treated all compartmental metabolites of an SSM the same. For example, taurine levels are significantly depleted in RYGB liver, but both cytosolic taurine (taur[c]) and peroxisomal taurine (taur[x]) are equally treated as SSMs in the Recon 2.1 model.

Module discovery using sub-graph search

The premise behind identifying sub-networks enriched with SSMs and SSRs is that significantly elevated or depleted biomolecules that are closely connected within a metabolic network provides evidence that RYGB is causing a perturbation to the module of reactions. Computationally, this is done by searching for random subgraphs in the Recon 2.1 reaction-centric graph of a specified size in a monte-carlo fashion and select sub-networks with a statistically higher density of SSRs and SSMs relative to the SSR/SSM density distribution for all random sub-graphs. The premise of identifying these sub-networks from a more complex network of human metabolism is highlighted in **Fig. 3a**. The random sub-graph generation begins by starting with the seed node in the reaction-centric matrix **G** and then randomly selecting an edge connected to the seed node. The target node connected to the randomly selected edge is then added to the sub-network. Subsequently, a randomly selected node from the now two-node sub-network is chosen as a point to expand the sub-network and another randomly selected edge connecting to that node is selected in a similar manner as described above. In this regard, the sub-network grows until it reaches a specified reaction

node size, in this case set to 20. The number of SSMs and SSRs in each sub-graph is then also computed.

For each of the 81 genes found to be significantly elevated or depleted between Sham and RYGB, 34 genes encode metabolic enzymes that catalyze at least one of the reactions described in the Recon 2.1 metabolic model. In total, 298 reactions in the model were catalyzed by one of the 34 metabolic genes found to be differentially expressed between the two experimental conditions. The central component of our target discovery methodology is to treat each of these reactions as a “seed” for a local network search algorithm to identify network modules with a high SSR and SSM density. The seed reaction, R_s , is used as a starting point to grow random connected sub-networks of 20 reaction nodes, which was determined to be a manageable module size for visual inspection. The distribution of maximum node-pair distance for random sub-networks of size 20 (**Supplementary Fig. 6**) shows that setting the module size to 20 is adequate to capture long range interactions spanning up to 9 reaction nodes. Since 99.88% of node-pair distances in **G** are less than or equal to 9, a module size of 20 can capture the most distant interactions in the network. The Monte-Carlo sub-graph detection algorithm is allowed to run for 1 hour and the composition of each module is stored along with the number of SSRs and SSMs. The algorithm is applied on the reaction centric matrix **G** and modules enriched with SSRs and SSMs are visualized using the bipartite representation **B** using Cytoscape network visualization software²³.

Modules detected

Several modules were discovered that exhibit regions within the metabolic network enriched with SSRs and SSMs, suggesting that the metabolic pathway captured by the module is influenced by RYGB. For example,

Figure 3 (a) Identifying sub-networks within a bipartite graph network of Recon 2.1 reveals modules either enriched or devoid of SSRs and SSMs. (b) Histogram for the distribution of the number of modular SSRs and SSMs and (c) the 90%, 95%, 99%, and 99.9% confidence bands.

a zoomed-in module in **Fig. 4a** reveals the relationship between the down-regulation of Baat and the depletion of taurine, the metabolite used to conjugate C24 bile acids. Baat is the enzyme that catalyzes the conjugation of taurine to choloyl-coA to form taurocholate, and the connection between the significantly depleted protein and metabolite is uncovered using computational module discovery in an unbiased fashion. Similarly, the module highlighted in **Fig. 4b** reveals a perturbation to “one-carbon folate metabolism” based on the differential expression of Mthfd1 and Ftcid as well as the increase in dihydrofolate levels. The network-based modularity approach also reveals lesser intuitive cofactor-based interactions within the local network neighborhood. For example, the module

featuring conjugated bile acid formation also (**Fig. 4a**) features the utilization CoA and other SSRs that consume or produce the cofactor. To further demonstrate the use of network-based modularity as a means to discover non-intuitive network interactions, several other modules are presented in **Supplementary Materials**, some of which highlight the production and consumption of NADPH, NADH, and other nucleotide triphosphates (**Supplementary Fig. 1–5**).

To determine if a module is significantly affected by RYGB, we compared the total SSMs and SSRs within the module to the multivariate distribution of modular SSMs and SSRs for approximately 10,000 random sub-networks of size 20. The histogram representing this distribution

Figure 4 Examples of detected modules featuring (a) Bile acid metabolism and (b) One-carbon metabolism. The SSRs and SSMs in each module are listed below the network diagrams.

(Fig. 3b) is heavily skewed where 48% of the modules contained zero SSRs. Since the distribution was non-normal, we numerically computed contour lines for the 90%, 95%, 99% and 99.9% bands (Fig. 4c) for which the specified percentage of modules lie to the left of the band. For example, the module representing bile acid conjugation (Fig. 4a) using taurine contained 8 SSPs and 7 SSMs, which falls above the 99% band suggesting that the module has a significantly high density of SSPs and SSMs relative to randomly connected sub-networks of size 20. Indeed, the sub-network size selected and the run-time for the Monte-Carlo are both parameters that can impact the modules that are uncovered. Quantitatively, we show that there is an inverse relationship between the run-time required to identify a known set of interactions between proteins and the size the module used for the algorithm (Supplementary Fig. 7). As such, while there is a run-time benefit to picking a larger module size, visually interpreting a sub-network of several hundred nodes would be too unwieldy.

DISCUSSION

In this work, we present a novel multi-omic interrogation of liver metabolism in a rat RYGB model by integrating SWATH proteomics and MRM metabolomics data to facilitate the discovery of biochemical mechanisms associated with weight loss and achievement of glycemic control. SWATH is now the state-of-the-art label-free proteomics platform that offers unparalleled coverage of an organism's proteome owing to the dynamic range and speed offered by AB Sciex Tripletof HRMS instrumentation. However, the application of this technology to translational medicine

research has been sparse and our utilization of the technique to quantify metabolic changes following RYGB *in vivo* using graph-based network analysis should serve as a generally applicable template for future multi-omic endeavors.

Our multi-omic analysis of rat liver metabolism following 3 months of either Sham or RYGB operations revealed significant differences in protein and metabolite levels, many of which corroborate observations made in the clinic. For example, the SWATH proteomics data revealed a strong perturbation to iron storage and transport in the liver, namely that ferritin (Fth1, responsible for iron storage) levels are significantly decreased following RYGB, which is consistent with the clinical finding that patients following RYGB undergo anemia²⁴. Intriguingly, increased serum ferritin has also been associated with insulin resistance via an inverse correlation to adiponectin levels²⁵. In addition, our module-detection algorithm found a perturbation to taurine-conjugated bile acid metabolism in the rat, which has also been observed in humans following RYGB. For example, Simonen *et al.* reported a decrease in fasting serum conjugated bile acids in RYGB patients, with an 82% reduction in taurine-conjugated bile acids²⁶. Moreover, they showed that the conjugated bile acid decrease correlated with glucose and lipid oxidation and proposed a mechanistic link to thyroid hormone regulation based on their observed increased mRNA expression of *DiO₂* in adipocytes. In contrast, Ahmad *et al.* reported post-prandial levels of taurine-conjugated bile acids in serum of lean, obese, and RYGB patients and found their levels to be higher in patients post-RYGB compared to obese patients²⁷. Different models of gastric bypass also seem to result in a perturbation to bile acid

metabolism, yet the trends appear opposite to what we observe following RYGB. For example, Wu *et al.* performed duodenal-jejunal bypass (DJB) and sleeve gastrectomy in diabetic rats and found that the expression of amino acid N-acyltransferase *Baat* was up-regulated with increased serum conjugated bile acid levels²⁸. As such, further investigation is warranted to understand the dynamics of *Baat* expression following RYGB and how that impacts the levels of conjugated bile acids either after fasting or post-prandial states.

The ultimate translational goal of interrogating rodent RYGB models using multi-omic approaches is to discover therapeutic targets for non-invasive obesity treatments. To this end, we report *Thrsp* and *Acot13* as two proteins down-regulated in RYGB animals (Fig. 3a), which have also been previously investigated for their impact on body weight regulation using knockout mouse models. Specifically, Kang *et al.* demonstrate that high fat diet fed *Them2*^{-/-} mice exhibited a 10% reduced body weight compared to high fat diet fed *Them2*^{+/+} mice²⁹ and Anderson *et al.* showed a 26/29 (male/female) % reduced body weight gain in a 11% fat by weight diet for *Thrsp* knockout mice³⁰. As a result, these targets may prove to be promising targets for hepatic knockdown using siRNA. However, further LOF/GOF experiments are required to determine if the down-regulation of these proteins can modulate body weight or if the observed protein expression following RYGB is instead a consequence of a more complex regulatory mechanism.

The module detection algorithm that was developed for this study provides an elegant integration of proteomics and metabolomics data to identify which metabolic pathways are impacted by the experimental perturbation. We find that modularity analysis can be advantageous to conventional pathway enrichment analysis (PEA) in uncovering lesser-intuitive interactions between statistically significant components. For example, conventional pathway analysis would have identified two SSPs, *Ftcd* and *Mthfd1*, as both belonging to “One carbon folate metabolism” (Supplementary Table 2). However, modularity analysis also showed that the SSP *Cyp2a2*, involved in detoxification, could also be connected to the pathway based on the shared consumption of NADPH (Fig. 4b). Network analysis also provides a framework for visually presenting the relationship between metabolomics and proteomics data in a way that would be challenging with PEA. For example, PEA on metabolomics data would have revealed taurine as an SSM involved in “Primary bile acid biosynthesis”; however, the relationship between taurine and the SSP enzyme *Baat* (Fig. 4a) may not be intuitive to a non-biochemist. Modularity analysis using metabolic networks precludes an additional manual curation step to piece together relationships between metabolites and proteins that would be required if only using conventional PEA methods.

One major challenge in interpreting the uncovered metabolic modules data lies in ascribing directional changes, or determining the up/down regulation of a set of closely related genes that resulted from the experimental condition. For example, the module capturing reactions from folate metabolism features increased levels of *Mthfd1* and dihydro-folate in RYGB compared to Sham, serving as multi-omic evidence that a tetrahydrofolate interconversion module is up-regulated (Fig. 4b). However, the decrease in *Ftcd*, which serves to replenish tetrahydrofolate from formiminoglutamate, obfuscates such a conclusion from being made without further experimentation. In this regard, the interpretation of modules enriched in SSRs and SSMs is limited to identifying perturbed regions of metabolic networks, and drawing conclusions about whole pathway directional changes is oftentimes a stretch due to conflicting evidence from multi-omic data. In another example, we find that while coenzyme A levels are decreased due to RYGB, two enzymes catalyzing reactions producing coA (*Baat*, *Acat2*) are differentially expressed in opposite directions (Supplementary Fig. 2), suggesting that the levels of proteins that consume coA are not driven solely by the metabolite pool.

The research workflow presented here, demonstrating a powerful multi-omic interrogation of rodent RYGB, will surely pave the way

for impactful future investigations. For example, in this study, we only performed the -omics analysis on a single time point, three months post-RYGB in just liver. A natural extension of this work will be to perform a time-course multi-omic analysis to study the dynamics of metabolic reprogramming in multiple organs (e.g., adipose tissue, small intestine, and muscle), to better capture the molecular underpinnings of the therapeutic benefits of the surgery. In addition, SWATH-based proteomics analysis of plasma samples and causal network analysis³¹ could enable the discovery of novel differentially expressed metabolites or proteins that may drive the observed perturbations to tissue-specific metabolic modules post-RYGB. Another angle to consider is the impact of the gut-microbiome compositional shifts following RYGB on metabolic outcomes. For example, Liou *et al.* demonstrated that RYGB in mice causes a significant shift in the gut-microbiome population, and that fecal transplants of RYGB treated animals into lean germ-free animals elicits a 5.0% body weight reduction¹¹. In this light, further examination of the gut-liver axis holds promise to discover microbial products regulate liver metabolism. Indeed, we also identify indole-3-carboxylic acid and phenylpyruvate to be significantly elevated RYGB liver extracts (Supplementary Table 2), both of which have previously been identified to be gut-microbiome derived using computational pathway analysis³².

In conclusion, we have demonstrated in this study a novel experimental and computational workflow as a technological advancement for the study of metabolic disease using a combination of rodent RYGB models and multi-omics approaches. We have applied this platform to interrogate the metabolic pathways that are altered in the liver following RYGB in rats, and identified several targets that may play a role in the regulation of hepatic energy homeostasis following the surgery. We expect that the combined computational and experimental framework presented here will further facilitate systematic investigations into the mechanisms that mediate the therapeutic benefits of the surgery, and ultimately, the discovery of novel therapeutics for the metabolic syndrome.

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SUPPLEMENTARY INFORMATION

Sections

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SUPPLEMENTARY METHODS

SWATH proteomics

Proteomics data using SWATH acquisition was collected on tissue biopsy (3 months post-surgery) protein extracts. Each biopsy was ground into powder using a mortar and pestle in liquid nitrogen, and

then reconstituted in 7 M urea, 2 M thiourea, 4% CHAPS, reduced and alkylated. Proteins were then precipitated with six volumes of acetone overnight after which they were washed twice with methanol: 41 g of each of the six RYGB and six Sham samples were pooled and digested on pellet with 2×3 g trypsin in 50 mM Tris pH 8 + 0.1% NaDOC. Peptides were fractionated into six fractions using increasing percentage of acetonitrile on a reverse phase solid phase extraction (SPE) column. The individual samples were treated identically, but were eluted with 75% acetonitrile. Each of the 12 fractions and 12 samples were then analyzed using LC-MS/MS. Acquisition was performed with a 5600 TripleTOF LC/MS-MS (AB Sciex, Foster City, CA) equipped with an electrospray interference with a 25 m iD capillary and coupled to an Eksigent UHPLC (Eksigent, Redwood City, CA). The acquisition and data processing was controlled using Analyst TF 1.6 software. The source voltage was set to 5.2 kV and maintained at 225°C, curtain gas was set as 27 psi, gas 1 at 12 psi, and gas 2 at 10 psi. Acquisition was performed in Information Dependent Acquisition (IDA) for the 2D protein database and in SWATH acquisition mode for the samples. Separation was performed on a reverse phase HALO C18-ES column 0.3 μ m i.d., 2.7 μ m particles, 150 mm long (Advance Materials Technology, Wilmington, DE) which was maintained at 60°C. Samples were injected by loop overfilling into a 5 μ L loop. For the 60 minute (2D database) or the 120 minute (samples) LC gradient, the mobile phase consisted of solvent A (0.2% v/v formic acid and 3% DMSO v/v in water) and solvent B (0.2% v/v formic acid and 3% DMSO in EtOH) at a flow rate of 3 μ L/min. Protein identification was performed with ProteinPilot V4.5 (AB Sciex) with the instrument pre-set for the AB Sciex 5600 TripleTOF and Iodoacetamide as cysteine alkylation as special factor.

Recon 2.1 network preparation

The Recon metabolic network was downloaded from humanmetabolism.org in a Matlab compatible .mat file, which comprised of a struct with reaction definitions, metabolite names with KEGG identification numbers, and a stoichiometric matrix. We chose to work with a human metabolic model since our findings in rat are only significant if applicable to target discovery for humans. Using this model, a bipartite graph network of both reaction and metabolite nodes was constructed in the form of a square connectivity matrix (**B**) of size $N + M$, where N is the number of reactions and M is the number of metabolites in the model respectively. With reactions listed before metabolites, the i th metabolite was assigned to the $(N + i)$ th row and column in **B**. A directed edge was assigned from a reaction node (R_i) to a metabolite node (M_j) if M_j is produced by the reaction R_i and is described by assigning a '1' to the entry $\mathbf{B}(i, N + i)$. Similarly, a directed edge was assigned from a M_i to R_i if the metabolite M_i is consumed by the reaction R_i and an entry '1' is assigned to $\mathbf{B}(N + i, i)$. All other matrix entries in **B** are otherwise assigned a value of '0'. If a reaction was assigned as reversible, both directions were considered for edge assignment. In this study, we included edges mediated through the production and consumption of metabolic cofactors such as ATP, NADH, NADPH, but excluded meaningless interactions based on inorganic ions such as H^+ . A complete list of which metabolites were considered for network edges is provided in **Supplementary Data 1**. From the bipartite representation of Recon 2, an $N \times N$ reaction-centric graph (**G**) was also constructed to enable module detection based on enzymes while metabolites are treated as a shared resource. In this case, an undirected edge between a reaction pair $[R_i, R_j]$ was assigned ($\mathbf{G}(i, j) = 1$) if a metabolite produced by R_i is consumed by R_j .

Supplementary Table 1 Significantly elevated or depleted liver proteins between Sham and RYGB.

Gene	SWATH AUC	Name/Enzyme Commission #	Function and Obesity/RYGB context
Tfrc p=0.002		Transferrin receptor protein 1	Involved with the up-regulation of iron acquisition molecule (TFRC) and may cause an increase in total hepatic iron content; the up-regulation of the gene may be a consequence of anemia; Tfrc gene polymorphisms are associated with Type II Diabetes Mellitus ^{S1} .
Fam65b p=0.002		Protein Fam65B	Transcriptional target of FOXO1 that regulates RhoA signaling for T lymphocyte signaling; implicated in the reduction of inflammation following bariatric surgery ^{S2} .
Scpep1 p=0.002		Retinoid-inducible serine carboxypeptidase	Contributes to the control of ET-1 regulation of vascular tone and hemodynamics ^{S3} .
Fth1 p=0.002		Ferritin heavy polypeptide 1	Important for iron homeostasis and ferroxidase activity; Taken up in the ferrous form and deposited as ferric hydroxide after oxidation; plays role in delivery of iron to cells ^{S4} .
Ociad2 p=0.009		OCIA domain-containing protein 2	Modulates gamma secretase which stimulates ameloid beta production ^{S5} .
Fdft1 p=0.028		Squalene synthase (SQS) (SS) (EC 2.5.1.21) (FPP:FPP farnesyltransferase) (Farnesyl-diphosphate farnesyltransferase)	Fdft1 is the enzyme which catalyzes the first step of cholesterol synthesis; In a Danish study looking at 18,014 individuals, Fdft1 failed to demonstrate an association with measures of obesity ^{S6} .

(Continued)

Supplementary Table 1 (Continued)

Gene	SWATH AUC	Name/Enzyme Commission #	Function and Obesity/RYGB context
7. Cpq p=0.026		Carboxypeptidase Q	Negative regulator of adipocyte differentiation; knock-down of Cpq in mature adipocytes leads to increased pro-adipogenic capacity of secreted fraction, implying this factor represents inhibitory molecules derived from mature adipocytes ^{S7} .
Asl p=0.009		Argininosuccinate lyase (ASAL) (EC 4.3.2.1) (Arginosuccinase)	Catalyzes fourth reaction in urea cycle, allows for breakdown of argininosuccinic acid to arginine and fumarate; involved in synthesis of arginine from ornithine and carbamoyl phosphate ^{S8} .
Glb1 p=0.022		No Gene Name	hydrolyzes β -galactose from glycoconjugates and is the origin of senescence-associated β -gal activity; Mutations in <i>Glb1</i> lead to deficiency in the activity of lysosomal β -galactosidase lipid accumulation ^{S9} .
Ebp p=0.041		3- β -hydroxysteroid-Delta(8),Delta(7)-isomerase	Enzyme involved with catalyzing an intermediate step in the conversion of lanosterol to cholesterol ^{S10} .
Al13 p=0.015		Alpha-1-inhibitor-3	Member of the macroglobulin superfamily of protease inhibitors; A negative acute phase protein which decreases significantly during an acute phase response; Studies have postulated that decreased levels of A1I3 in the blood would increase the activities of proteases causing non-specific breakdown of proteins and damage of intercellular tissue which would lead to loss of body weight ^{S11} .
Ftcd p=0.015		Formimidoyltransferase-cyclodeaminase	Involved in folate metabolism (THF); The gene encodes the enzyme formiminotransferase cyclodeaminase which is a metabolism enzyme that links histidine catabolism to folate metabolism ^{S12} .

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Supplementary Table 1 (Continued)

Gene	SWATH AUC	Name/Enzyme Commission #	Function and Obesity/RYGB context
Igkc p=0.002		Immunoglobulin Kappa Constant Region	Strong prognostic marker in several human solid tumors, including breast cancer; mechanism is not entirely known ^{S13} .
Plin2 p=0.013		Perilipin 2	Increased expression <i>Plin2</i> leads to accumulation of cellular lipids; Overexpression of the gene affects enzymes involved in lipolysis and lipogenesis; Cells overexpressing <i>Plin2</i> had elevated levels of triacylglycerols, fatty acids, cholesteryl esters, and phospholipids; Studies have shown that <i>Plin2</i> deficiency results in reduced lipid droplet size and number ^{S14} .
Aass p=0.002		Alpha-aminoadipic semialdehyde synthase, mitochondrial	Involved in lysine metabolism; Saccharopine in the pathway is oxidized to α -aminoadipic semialdehyde and glutamate by saccharopine dehydrogenase ^{S15} .
Acat2 p=0.004		Acetyl-coA acetyltransferase 2	Involved in cholesterol metabolism, catalyzing the conversion of acetyl-CoA to acetoacetyl-coA; ACAT2-selective inhibitors have been associated with effective cholesterol-lowering or anti-atherosclerotic agents ^{S16} .
Tymp p=0.026		Thymidine phosphorylase	Encodes for a growth factor involved in cell proliferation and angiogenesis; Involved in nucleotide synthesis; Catalyzes the reversible phosphorolysis of thymidine which regulates the amount of nucleosides in cells necessary to maintain a balanced pool of deoxyribonucleoside triphosphate for replication and repair of DNA ^{S17} .
Fabp7 p=0.015		Fatty acid binding protein 7	Associated with fatty acid metabolism; Involved in hepatic steatosis ^{S18} .

(Continued)

Supplementary Table 1 (Continued)

Gene	SWATH AUC	Name/Enzyme Commission #	Function and Obesity/RYGB context
Map1 p=0.002		Microtubule associated protein 1	Predominately expressed in neurons and are involved in the formation and development of axons and dendrites. Contribute to microtubule-stabilizing activity ^{S19} .
Hao1 p=0.026		Hydroxyacid oxidase 1	Associated with high fatty acid oxidase activity in the liver and isolates and characterizes glycolate oxidase from human liver; Hao1 is down-regulated in different models of in vivo oxidative stress; The down-regulation of Hao1 expression during oxidative stress may represent a mechanism to prevent excessive H2O2 ^{S20} .
Ech1 p=0.026		Delta(3,5)-Delta(2,4) dienoyl-CoA isomerase	Second enzyme in the pathway of fatty acid degradation via β -oxidation ^{S21} .
Got1 p=0.002		Aspartate aminotransferase	TCA cycle: Converts glutatmine-derived aspartate into oxaloacetate (OAA) ^{S22} .
Ephx2 p=0.009		Bifunctional epoxide hydrolase 2	Associated with altering levels of epoxyeicosatrienoic acids; Disruption of Ephx2 significantly attenuates hepatic steatosis ^{S23} .
Slc27a5 p=0.015		Bile acyl-CoA sythetase	Hepatic BA-CoA ligase involved in re-conjugating bile acids during enterohepatic bile acid recycling; Slc27a5 methylation signifcantly altered in non-alcoholic fatty liver disease ^{S24} .

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Supplementary Table 1 (Continued)

Gene	SWATH AUC	Name/Enzyme Commission #	Function and Obesity/RYGB context
Gk p=0.004		Glycerol kinase	Uptake and metabolism of glycerol; Regulates hepatic glucose production, glycerol gluconeogenesis axis ^{S25} .
Ndufs2 p=0.026		NADH dehydrogenase	Involved with the mitochondrial respiratory chain, the main source of free radicals ^{S26} .
Bhmt p=0.004		Betaine-homocysteine S-methyltransferase	Involved in regulation of homocysteine metabolism; Converts homocysteine to methionine using betaine and S-methylmethionine, respectively, as methyl donor substrates ^{S27} .
Thrsp p=0.015		Thyroid hormone-inducible protein	Encodes 150 amino acid proteins and regulates fatty acid synthesis in tissue; Studies have shown that the Thrsp gene deletion affects body weight and glucose tolerance; Associated with increased insulin sensitivity; Hepatic Thrsp gene silencing attenuates fatty liver phenotype ^{S28} .
Pipox p=0.041		L-pipecolic acid oxidase	Major lipogenic enzyme in the sarcosine metabolism pathway; Converts sarcosine to glycine by oxidative demethylation ^{S29} .
Aldh8a1 p=0.041		Aldehyde dehydrogenase family 8 member A1	Converts 9-cis-retinal to 9-cis retinoic acid which is a ligand of retinoid X receptor (RXR), one of the master regulators of gene expression in the liver ^{S30} .

(Continued)

Supplementary Table 1 (Continued)

Gene	SWATH AUC	Name/Enzyme Commission #	Function and Obesity/RYGB context
Cat p=0.009		Catalase	Responsible for reducing H2O2 to H2O in the peroxisomes; CAT is easily induced by oxidative stress in cells ^{S31} .
Acy3 p=0.041		N-acyl-aromatic-L-amino acid amidohydrolase	Involved in the hydrolysis of N-acylated or N-acetylated amino acids ^{S32} .
Gstt3 p=0.026		Protein Gstt3	Mostly expressed in the liver and plays a role in Glutathione metabolism; Significantly down-regulated in NAFLD ^{S33} .
Rpl23 p=0.004		60S ribosomal protein L23	Ribosomal protein that interacts with MDM2 to inhibit its E3 ligase function, which results in stabilizing and activating p53 (RPDM253 signaling pathway) ^{S34} .
Ctsc p=0.026		Dipeptidyl peptidase	Encodes for Dipeptidyl-peptidase I which removes dipeptides from the amino-terminus of protein substrates; Associated with immunity and inflammatory tasks such as activating phagocytic and T-lymphocytes cells involved with eliminating pathogens ^{S35} .
Idh2 p=0.015		Isocitrate dehydrogenase	Encodes for isoforms of the nicotinamide adenine dinucleotide phosphate-dependent isocitrate dehydrogenase; Involved in TCA cycle; Plays a major role in insulin sensitivity and fuel metabolism ^{S36} .

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Supplementary Table 1 (Continued)

Gene	SWATH AUC	Name/Enzyme Commission #	Function and Obesity/RYGB context
Enpp3 p=0.028		Ectonucleotide pyrophosphatase	Type II transmembrane glycoprotein with a small N-terminal intracellular domain, a single-spanning transmembrane domain, and a large catalytic C-terminal extracellular domain; Involved in purine and pyrimidine metabolism ^{S37} .
Mut p=0.002		Methylmalonyl-coA mutase	Cobalamin (B12)-dependent enzyme that catalyzes the isomerization of Methylmalonyl-CoA to succinyl-CoA which is required in the catabolism of odd fatty acids and certain amino acids ^{S38} .
Hykk p=0.026		Hydroxylysine kinase	Involved in lysine catabolism and proline and tryptophan catabolism ^{S39} .
Hsd17b13 p=0.015		Hydroxysteroid 17-beta dehydrogenase 13	HSD17B13 has regulatory miRNAs, miR-375 and miR-154-5p which may be associated with levels of triglycerides; HSD17B13 has been shown to be expressed in adipose tissue, and miR-375 is known to be involved in regulation of glucose and lipid metabolism ^{S40} .
Vim p=0.041		Vimentin	An Intermediate filament protein in mesenchymal cells; Significantly increased in hepatic steatosis ^{S41} .
Esd p=0.015		S-formylglutathione hydrolase	Involved in the detoxification of formaldehyde; Reduced ESD activities have been linked to the susceptibility of pathological conditions such as toxic liver cirrhosis ^{S42} .

(Continued)

Supplementary Table 1 (Continued)

Gene	SWATH AUC	Name/Enzyme Commission #	Function and Obesity/RYGB context
Nme2 p=0.041		Nucleoside diphosphate kinase 2	Encodes the B subunit of the nucleoside diphosphate (NDP) kinase; Studies shows that NME2 binds the telomere repeat binding factor 2 in the nucleus to reduce telomerase activity ^{S43} .
Adk p=0.041		Adenosine kinase	Nucleoside kinase that catalyzes the phosphorylation of the purine nucleoside adenosine at the 5position in an ATP-dependent manner; vital enzyme cofactor for FADH, NAD(P)H, and coenzyme A, and its biochemical effects also include activation of adenylate cyclase, inhibition of pyrimidine biosynthesis, and diminution of phosphoribosyl pyrophosphate synthesis ^{S44} .
Aldh9a1 p=0.009		Aldehyde dehydrogenase	Encodes γ-trimethylaminobutyraldehyde dehydrogenase which plays a role in the metabolism of γ-aminobutyraldehyde and aminoaldehydes derived from polyamines ^{S45} .
Hspa9 p=0.015		Heat shock protein 9	HSPA9, a member of the heat shock protein (HSP) 70 family, was first identified as human mitochondrial hsp75 and has important roles in stress response, glucose regulation, cell proliferation, differentiation, and tumorigenesis ^{S46} .
Cyp2a2 p=0.026		Cytochrome p450	Catalyzes formation of several metabolites, including 15- alpha- hydroxytestosterone; Involved in retinol metabolism ^{S47} .
Prpsap2 p=0.015		Phosphoribosyl pyrophosphate synthetase-associated protein 2	Phosphoribosylpyrophosphate (PRPP) is a primary substrate and a critical control factor for de novo synthesis of purine and pyrimidine nucleotides, histidine and tryptophan, and NAD ^{S48} .

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Supplementary Table 1 (Continued)

Gene	SWATH AUC	Name/Enzyme Commission #	Function and Obesity/RYGB context
Snrpd3 p=0.015		Small nuclear ribonucleoprotein D1	- SNRPD3 is a core component of the spliceosome and VCPn ATPase of the endoplasmic reticulum that prevents mutations caused by DNA damage ^{S49} .
Ndufv2 p=0.002		NADH dehydrogenase	Encodes the 24 kDa subunit of complex I, and is involved in electron transfer; NDUFV2 protein is synthesized in the cytosol on free ribosomes as a precursor protein and is transported across mitochondrial membranes ^{S50} .
Aco2 p=0.026		Aconitase 2	Important regulatory enzyme of the TCA cycle; The aco2 gene produces two kinds of proteins, one with the aconitase domain only that is targeted almost exclusively to mitochondria, and the other with both aconitase and bL21 domains, which are targeted to both nucleus and mitochondria; Down regulated in the liver of high-fat diet-fed animals ^{S51} .
Fmo1 p=0.015		Flavin containing monooxygenase 1	FMO1 catalyzes the oxidation of trimethylamine to its N-oxide ^{S52} .
Aadat p=0.009		Amino adipate aminotransferase	Involved in the conversion of α -amino adipate to α -keto adipate by α -amino adipate aminotransferase (AADA) which is an intermediate step in lysine degradation ^{S53} .
Actn4 p=0.041		Actinin alpha 4	Transcriptional coactivator with protein-protein interactions among actinin-4, class II histone deacetylases, and myocyte enhancer factor 2s (MEF2s); Actn4 expression is essential for the transcriptional activity of NF- κ B in the presence of TNF- α ^{S54} .

(Continued)

Supplementary Table 1 (Continued)

<div><div><div>● SHAM</div><div>● RYGB</div></div></div>					
Gene	SWATH AUC	Name/Enzyme Commission #	Function and Obesity/RYGB context		
Baat p=0.041		Bile acid-CoA:amino acid N-acyltransferase	The enzyme is involved with bile acid biosynthesis by mediating conjugation of bile acids with the amino acids glycine and taurine. Implicated in NAFLD ^{S55} .		
LOC100362366 p=0.041		40S ribosomal protein S17-like	Primary rRNA binding protein which is thought to be involved in recognizing termination codons ^{S56} .		
Slc37a4 p=0.041		Glucose-6-phosphate transporter	The gene functions as a transporter for a monophosphate ester by structural comparison to various types of bacterial monophosphate ester transporter proteins ^{S57} .		
Cpox p=0.026		Coproporphyrinogen oxidase	A mitochondrial enzyme involved in the porphyrin metabolism pathway and plays a role in oxidizing coproporphyrinogen III to produce protoporphyrinogen ^{S58} .		
Cyp1a2 p=0.026		Cytochrome p450	This gene works in the liver by detoxifying various compounds. Altered in NAFLD ^{S59} .		
Iyd p=0.041		Iodotyrosine deiodinase	The flavoprotein iodotyrosine deiodinase salvages iodide from mono- and diiodotyrosine which are by-products of thyroid hormone synthesis ^{S60} .		

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Supplementary Table 1 (Continued)

Gene	SWATH AUC	Name/Enzyme Commission #	Function and Obesity/RYGB context
Acot13 p=0.035		Acyl-CoA thioesterase 13	The enzyme is involved in lipid biosynthesis and fatty acid synthesis. It is an interacting partner for phosphatidylcholine transfer protein which is a highly specific intracellular lipid-binding protein. ^{S61}
Ywhab p=0.035		Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, theta	The enzyme regulates the activity of ChREBP (carbohydrate response element-binding protein) which regulates expression of genes involved in hepatic glycolysis and lipogenesis ^{S62} .
Gss p=0.041		Glutathione synthetase	Involved in gamma-glutamyl cycle which produces glutathione molecules and is important to protect cells from oxidative damage and plays a role in membrane transport of amino acids ^{S63} .
Gys2 p=0.009		Glycogen synthase 2	Predominately expressed in the liver and is the rate-limiting enzyme for glycogenesis ^{S64} .
Adhfe1 p=0.026		Hydroxyacid-oxoacid transhydrogenase	Encodes hydroxyacid-oxoacid transhydrogenase which is responsible for the oxidation of 4-hydroxybutyrate to succinate semialdehyde. Studies have shown that Adhfe1 transcript dysregulation in obese adipose tissue and obese kidney influences obesity parameters ^{S65} .
Aldh3a2 p=0.026		Aldehyde dehydrogenase	The gene is a microsomal enzyme that catalyzes the oxidation of medium- and long-chain aliphatic aldehydes to fatty acids ^{S66} .

(Continued)

Supplementary Table 1 (Continued)

Gene	SWATH AUC	Name/Enzyme Commission #	Function and Obesity/RYGB context
Echdc3 p=0.026		Enoyl coA hydratase	involved in fatty acid synthesis in adipocytes. Also presumed to be involved in beta oxidation ^{S67} .
Acsf2 p=0.002		Fatty-acyl-coA synthetase	Involved in activating fatty acids by forming a thioester bond with CoA. Studies have demonstrated that a change in gene expression of ACSF2 is associated with lowering of abdominal adiposity and blood triacylglycerol levels in rats ^{S68} .
Acnat2 p=0.026		Acyl-coenzyme A amino acid N-acyltransferase 2	It is involved in the taurine conjugation of unsaturated acyl-CoAs ^{S69} .
Hyi p=0.026		hydroxypyruvate isomerase	Catalyzes the reversible isomerization between hydroxypyruvate and 2-hydroxy-3-oxopropanoate ^{S70} .
LOC103692171 p=0.015		n/a	n/a
Scly p=0.041		Selenocysteine lyase	Selenocysteine lyase catalyzes the decomposition of selenocysteine into alanine and selenide. Mice lacking Scly have been shown to develop metabolic syndrome, hyperinsulinemia, hyperleptinemia, and hypercholesterolemia ^{S71} .

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Supplementary Table 1 (Continued)

Gene	SWATH AUC	Name/Enzyme Commission #	Function and Obesity/RYGB context
Hp p=0.026		Haptoglobin	An acute phase protein that prevents both iron loss and kidney damage during hemolysis ^{S72} .
Eef1d p=0.041		Eukaryotic translation elongation factor 1 delta	Necessary for the regulation of cyclin D1 and vimentin. Studies have demonstrated a decrease in EEF1D levels results in a decrease in cell cycling and proliferation ^{S73} .
IgG-2a p=0.041		Immunoglobulin G 2a	The gene is involved in immune response such as macrophage activation ^{S74} .
Mthfd1 p=0.041		C-1-tetrahydrofolate synthase	It is a tri-functional folate-metabolizing enzyme with two domains. The synthetase domain catalyzes the synthesis of 10-formyltetrahydrofolate (formylTHF) from tetrahydrofolate (THF) and formate. The DC domain interconverts 10-formylTHF and 5,10-methyleneTHF ^{S75} .
LOC103691744 p=0.041		n/a	n/a
Psm12 p=0.026		26S proteasome non-ATPase regulatory subunit 12	PSMD12 is a subunit of the 26S proteasome without ATPase, which contains a PINT domain shown to interact with several basal transcription factors ^{S76} .

(Continued)

Supplementary Table 1 (Continued)

Gene	SWATH AUC	Name/Enzyme Commission #	Function and Obesity/RYGB context
Cmbl p=0.002		Carboxymethylenebutenolidase	CMBL has been shown to be a primary bioactivating enzyme in the liver and intestine. CMBL hydrolyzes the ester bond of the prodrug type xenobiotics ^{S77} .
Pcbp2 p=0.015		Poly (rC)-binding protein 2	PCBPs regulate gene expression at transcription, mRNA processing, mRNA stabilization, and translation. Involved in regulation of hepatic insulin sensitivity ^{S78} .
Prosc p=0.041		Proline synthetase cotranscribed	The enzyme is ubiquitously expressed in human tissues. The gene product is likely to be a soluble cytoplasmic protein but its function remains to be determined ^{S79} .

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Supplementary Table 2 ^AMetabolic pathways or modules described in the KEGG Database; ^BNumber of SSPs between Sham and RYGB KEGG pathway; ^CList of SSPs.

KEGG Pathway ^A	# of SSPs ^B	List of SSPs ^C
Lysine degradation	8	Aass, Acat2, Pipox, Hykk, Aldh9a1, Hspa9, Aadat, Aldh3a2
Tryptophan metabolism	6	Acat2, Aldh9a1, Hspa9, Aadat, Cyp1a2, Aldh3a2
Peroxisome	6	Hao1, Ech1, Ephx2, Pipox, Idh2, Baat
Biosynthesis of amino acids	5	Asl, Got1, Idh2, Aco2, Aadat
Valine, leucine and isoleucine degradation	5	Acat2, Mut, Aldh9a1, Hspa9, Aldh3a2
Glyoxylate and dicarboxylate metabolism	5	Acat2, Hao1, Mut, Aco2, Hyi
Histidine metabolism	4	Ftcd, Aldh9a1, Hspa9, Aldh3a2
Fatty acid degradation	4	Acat2, Aldh9a1, Hspa9, Aldh3a2
Pyruvate metabolism	4	Acat2, Aldh9a1, Hspa9, Aldh3a2
Arginine and proline metabolism	4	Got1, Aldh9a1, Hspa9, Aldh3a2
2-Oxocarboxylic acid metabolism	4	Got1, Idh2, Aco2, Aadat
Glycerolipid metabolism	4	Gk, Aldh9a1, Hspa9, Aldh3a2
PPAR signaling pathway	3	Fabp7, Slc27a5, Gk
Cysteine and methionine metabolism	3	Got1, Bhmt, Gss
Purine metabolism	3	Enpp3, Nme2, Adk
Epstein-Barr virus infection	3	Vim, Ywhab, Psmd12
Glycolysis/Gluconeogenesis	3	Aldh9a1, Hspa9, Aldh3a2
Ascorbate and aldarate metabolism	3	Aldh9a1, Hspa9, Aldh3a2
beta-Alanine metabolism	3	Aldh9a1, Hspa9, Aldh3a2
Steroid biosynthesis	2	Fdft1, Ebp
Arginine biosynthesis	2	Asl, Got1
Alanine, aspartate and glutamate metabolism	2	Asl, Got1
Lysosome	2	Glb1, Ctsc
One carbon pool by folate	2	Ftcd, Mthfd1
Propanoate metabolism	2	Acat2, Mut
Pyrimidine metabolism	2	Tymp, Nme2
Primary bile acid biosynthesis	2	Slc27a5, Baat
Insulin resistance	2	Slc27a5, Gys2
Bile secretion	2	Slc27a5, Baat
Oxidative phosphorylation	2	Ndufs2, Ndufv2
Non-alcoholic fatty liver disease (NAFLD)	2	Ndufs2, Ndufv2
Alzheimer's disease	2	Ndufs2, Ndufv2
Parkinson's disease	2	Ndufs2, Ndufv2
Huntington's disease	2	Ndufs2, Ndufv2
Glycine, serine and threonine metabolism	2	Bhmt, Pipox
Citrate cycle (TCA cycle)	2	Idh2, Aco2
Glutathione metabolism	2	Idh2, Gss
Starch and sucrose metabolism	2	Enpp3, Gys2
Retinol metabolism	2	Cyp2a2, Cyp1a2
Systemic lupus erythematosus	2	Snrpd3, Actn4
Drug metabolism - cytochrome P450	2	Fmo1, Cyp1a2
Viral carcinogenesis	2	Actn4, Ywhab

Supplementary Table 3 Individual metabolite levels based on normalized peak areas comparing Sham and RYGB for SSMs.

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Supplementary Table 3 (Continued)

(Continued)

Supplementary Table 3 (Continued)

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Supplementary Table 4 ^AMetabolic pathways or modules described in the KEGG Database; ^BNumber of SSMs between Sham and RYGB KEGG pathway; ^CList of SSMs.

KEGG Pathway ^A	Num of SSMs ^B	List of SSMs ^C
Pyrimidine metabolism	12	carbamoyl phosphate, dUMP, dCDP, UDP, dCTP, CTP, UTP, UDP-D-glucose, thymine, glutamine, dTMP, guanidoacetic acid
Biosynthesis of alkaloids derived from ornithine, lysine and nicotinic acid	11	succinate, quinolinate, D-glyceraldehyde-3-phosphate, hexose-phosphate, glucose-6-phosphate, fructose-6-phosphate, acetyl-CoA, DL-pipecolic acid, aspartate, glutamate, 2-keto-isovalerate
Purine metabolism	10	carbamoyl phosphate, cyclic-AMP, ADP, dGDP, dATP, ATP, dGTP, glycine, glutamine, maleic acid
Glutathione metabolism	10	pyroglutamic acid, glutathione, NADPH, acetyl-CoA, glycine, glutamate, glutathione, glutathione disulfide, NADPH, maleic acid
ABC transporters	10	taurine, glutathione, trehalose-sucrose, cellobiose, glycine, aspartate, glutamine, glutamate, biotin, maleic acid
Glycolysis/Gluconeogenesis	9	lactate, dihydroxy-acetone-phosphate, D-glyceraldehyde-3-phosphate, hexose-phosphate, glucose-1-phosphate, glucose-6-phosphate, fructose-6-phosphate, thiamine pyrophosphate, fumarate
Biosynthesis of plant hormones	9	succinate, D-glyceraldehyde-3-phosphate, hexose-phosphate, glucose-6-phosphate, fructose-6-phosphate, trans, trans-farnesyl diphosphate, acetyl-CoA, aspartate, fumarate
Neuroactive ligand-receptor interaction	9	taurine, UDP, ADP, UTP, ATP, glycine, 4-aminobutyrate, aspartate, fumarate
Central carbon metabolism in cancer	9	lactate, succinate, acetyl-CoA, glycine, asparagine, aspartate, glutamine, glutamate, fumarate
Alanine, aspartate and glutamate metabolism	8	succinate, carbamoyl phosphate, 4-aminobutyrate, asparagine, aspartate, glutamine, glutamate, uracil
Starch and sucrose metabolism	8	hexose-phosphate, glucose-1-phosphate, glucose-6-phosphate, fructose-6-phosphate, trehalose-sucrose, cellobiose, UDP-D-glucose, uracil
Biosynthesis of phenylpropanoids	8	succinate, D-glyceraldehyde-3-phosphate, hexose-phosphate, glucose-6-phosphate, fructose-6-phosphate, acetyl-CoA, 7,8-dihydrofolate, uracil
Biosynthesis of alkaloids derived from shikimate pathway	8	succinate, D-glyceraldehyde-3-phosphate, hexose-phosphate, glucose-6-phosphate, fructose-6-phosphate, acetyl-CoA, tyrosine, uracil
Biosynthesis of alkaloids derived from terpenoid and polyketide	8	succinate, D-glyceraldehyde-3-phosphate, hexose-phosphate, glucose-6-phosphate, fructose-6-phosphate, trans, trans-farnesyl diphosphate, acetyl-CoA, uracil
Biosynthesis of alkaloids derived from histidine and purine	7	succinate, D-glyceraldehyde-3-phosphate, hexose-phosphate, glucose-6-phosphate, fructose-6-phosphate, acetyl-CoA, glycerate
Galactose metabolism	6	dihydroxy-acetone-phosphate, D-glyceraldehyde-3-phosphate, glucose-1-phosphate, glucose-6-phosphate, trehalose-sucrose, acetoacetate
Arginine biosynthesis	6	carbamoyl phosphate, aspartate, glutamine, glutamate, citrulline, acetoacetate
Amino sugar and nucleotide sugar metabolism	6	hexose-phosphate, glucose-1-phosphate, glucose-6-phosphate, fructose-6-phosphate, UDP-D-glucose, acetoacetate
Glyoxylate and dicarboxylate metabolism	6	succinate, acetyl-CoA, glycine, glutamine, glutamate, acetoacetate
Propanoate metabolism	6	lactate, 2-oxobutanoate, succinate, dihydroxy-acetone-phosphate, acetyl-CoA, acetoacetate
Aminoacyl-tRNA biosynthesis	6	glycine, asparagine, aspartate, glutamine, glutamate, acetoacetate
Biosynthesis of terpenoids and steroids	6	succinate, D-glyceraldehyde-3-phosphate, hexose-phosphate, fructose-6-phosphate, trans, trans-farnesyl diphosphate, acetoacetate
Thyroid hormone synthesis	6	glutathione, cyclic-AMP, NADPH, glutathione, glutathione disulfide, acetoacetate
Glucagon signaling pathway	6	lactate, succinate, glucose-1-phosphate, glucose-6-phosphate, cyclic-AMP, acetoacetate
Protein digestion and absorption	6	glycine, asparagine, aspartate, glutamine, glutamate, acetoacetate
Glycolysis (Embden-Meyerhof pathway), glucose = pyruvate	5	dihydroxy-acetone-phosphate, D-glyceraldehyde-3-phosphate, hexose-phosphate, glucose-6-phosphate, 2-oxobutanoate
Pyrimidine deoxyribonucleotide biosynthesis, CDP/CTP => dCDP/dCTP,dTDP/dTTP	5	dUMP, dCDP, dCTP, CTP, 2-oxobutanoate
Fructose and mannose metabolism	5	lactate, dihydroxy-acetone-phosphate, D-glyceraldehyde-3-phosphate, hexose-phosphate, 2-oxobutanoate
Cysteine and methionine metabolism	5	2-oxobutanoate, S-ribosyl-L-homocysteine-nega, glutathione, aspartate, 2-oxobutanoate
Phenylalanine metabolism	5	succinate, phenylpyruvate, acetyl-CoA, tyrosine, 2-oxobutanoate
beta-Alanine metabolism	5	quinolinate, acetyl-CoA, 4-aminobutyrate, aspartate, 2-oxobutanoate

(Continued)

Supplementary Table 4 (Continued)

KEGG Pathway ^A	Num of SSMs ^B	List of SSMs ^C
Butanoate metabolism	5	succinate, acetyl-CoA, 4-aminobutyrate, glutamate, 2-oxobutanoate
GABAergic synapse	5	succinate, cyclic-AMP, 4-aminobutyrate, glutamine, 2-oxobutanoate
Bile secretion	5	glutathione, cyclic-AMP, acetyl-CoA, glutathione, 2-oxobutanoate
Vitamin digestion and absorption	5	thiamine pyrophosphate, coenzyme A, biotin, coenzyme A-posi, 2-oxobutanoate
Gluconeogenesis, oxaloacetate = fructose-6P	4	dihydroxy-acetone-phosphate, D-glyceraldehyde-3-phosphate, hexose-phosphate, lactate
Pentose phosphate pathway (Pentose phosphate cycle)	4	D-glyceraldehyde-3-phosphate, hexose-phosphate, glucose-6-phosphate, lactate
Urea cycle	4	carbamoyl phosphate, aspartate, citrulline, lactate
Citrate cycle (TCA cycle)	4	succinate, thiamine pyrophosphate, acetyl-CoA, lactate
Pentose phosphate pathway	4	D-glyceraldehyde-3-phosphate, hexose-phosphate, glucose-6-phosphate, lactate
Glycine, serine and threonine metabolism	4	2-oxobutanoate, guanidoacetic acid, glycine, lactate
Arginine and proline metabolism	4	guanidoacetic acid, 4-aminobutyrate, glutamate, lactate
Histidine metabolism	4	Imidazoleacetic acid, aspartate, histidinol, lactate
Cyanoamino acid metabolism	4	glycine, asparagine, aspartate, lactate
Pyruvate metabolism	4	lactate, succinate, thiamine pyrophosphate, lactate
Methane metabolism	4	dihydroxy-acetone-phosphate, D-glyceraldehyde-3-phosphate, acetyl-CoA, lactate
Thiamine metabolism	4	D-glyceraldehyde-3-phosphate, thiamine pyrophosphate, glycine, lactate
Nicotinate and nicotinamide metabolism	4	quinolate, dihydroxy-acetone-phosphate, 4-aminobutyrate, lactate
Pantothenate and CoA biosynthesis	4	dephospho-CoA-nega, coenzyme A, aspartate, lactate
Two-component system	4	succinate, aspartate, glutamine, lactate
cAMP signaling pathway	4	lactate, succinate, cyclic-AMP, lactate
Synaptic vesicle cycle	4	ATP, glycine, 4-aminobutyrate, lactate
Cholinergic synapse	4	cyclic-AMP, coenzyme A, acetyl-CoA, lactate
Parkinson's disease	4	cyclic-AMP, ADP, ATP, lactate
Citrate cycle (TCA cycle, Krebs cycle)	3	succinate, acetyl-CoA, pyruvate
GABA (gamma-Aminobutyrate) shunt	3	succinate, 4-aminobutyrate, pyruvate
Reductive citrate cycle (Arnon-Buchanan cycle)	3	succinate, acetyl-CoA, pyruvate
Dicarboxylate-hydroxybutyrate cycle	3	succinate, acetyl-CoA, pyruvate
3-Hydroxypropionate bi-cycle	3	succinate, acetyl-CoA, pyruvate
Pentose and glucuronate interconversions	3	dihydroxy-acetone-phosphate, glucose-1-phosphate, pyruvate
Fatty acid degradation	3	coenzyme A, acetyl-CoA, pyruvate
Oxidative phosphorylation	3	succinate, ADP, pyruvate
Valine, leucine and isoleucine degradation	3	thiamine pyrophosphate, acetyl-CoA, pyruvate
Lysine degradation	3	acetyl-CoA, glycine, pyruvate
Carbapenem biosynthesis	3	coenzyme A, glutamate, pyruvate
Taurine and hypotaurine metabolism	3	taurine, acetyl-CoA, pyruvate
Inositol phosphate metabolism	3	dihydroxy-acetone-phosphate, D-glyceraldehyde-3-phosphate, pyruvate
Terpenoid backbone biosynthesis	3	D-glyceraldehyde-3-phosphate, trans, trans-farnesyl diphosphate, pyruvate
Nitrogen metabolism	3	carbamoyl phosphate, glutamine, pyruvate
HIF-1 signaling pathway	3	lactate, ATP, pyruvate
AMPK signaling pathway	3	glucose-6-phosphate, ADP, pyruvate
Platelet activation	3	cyclic-AMP, ADP, pyruvate
Retrograde endocannabinoid signaling	3	cyclic-AMP, 4-aminobutyrate, pyruvate
Glutamatergic synapse	3	cyclic-AMP, glutamine, pyruvate
Taste transduction	3	cyclic-AMP, trehalose-sucrose, pyruvate
Renin secretion	3	cyclic-AMP, ADP, pyruvate

Supplementary Table 4 (Continued)

KEGG Pathway ^A	Num of SSMs ^B	List of SSMs ^C
Mineral absorption	3	glycine, asparagine, pyruvate
Cocaine addiction	3	cyclic-AMP, glutamate, pyruvate
Amphetamine addiction	3	cyclic-AMP, glutamate, pyruvate
Alcoholism	3	cyclic-AMP, glutamate, pyruvate
Ethylmalonyl pathway	2	acetyl-CoA, glycolate
Hydroxypropionate-hydroxybutyrate cycle	2	acetyl-CoA, glycolate
Photorespiration	2	glycine, glycolate
Methylaspartate cycle	2	acetyl-CoA, glycolate
Ascorbate and aldarate metabolism	2	D-glucarate, glycolate
Primary bile acid biosynthesis	2	taurine, glycolate
Puromycin biosynthesis	2	ATP, glycolate
Monobactam biosynthesis	2	aspartate, glycolate
Valine, leucine and isoleucine biosynthesis	2	2-oxobutanoate, glycolate
Lysine biosynthesis	2	acetyl-CoA, glycolate
Tyrosine metabolism	2	succinate, glycolate
Benzoate degradation	2	acetyl-CoA, glycolate
Tryptophan metabolism	2	quinolinate, glycolate
Phenylalanine, tyrosine and tryptophan biosynthesis	2	phenylpyruvate, glycolate
D-Glutamine and D-glutamate metabolism	2	glutamine, glycolate
Glycerolipid metabolism	2	dihydroxy-acetone-phosphate, glycolate
C5-Branched dibasic acid metabolism	2	2-oxobutanoate, glycolate
Sulfur metabolism	2	succinate, glycolate
Tropane, piperidine and pyridine alkaloid biosynthesis	2	phenylpyruvate, glycolate
Phosphotransferase system (PTS)	2	trehalose-sucrose, glycolate
Calcium signaling pathway	2	cyclic-AMP, glycolate
FoxO signaling pathway	2	ADP, glycolate
Lysosome	2	ADP, glycolate
Gap junction	2	cyclic-AMP, glycolate
Circadian entrainment	2	cyclic-AMP, glycolate
Long-term potentiation	2	cyclic-AMP, glycolate
Dopaminergic synapse	2	cyclic-AMP, glycolate
Inflammatory mediator regulation of TRP channels	2	cyclic-AMP, glycolate
Insulin secretion	2	cyclic-AMP, glycolate
Estrogen signaling pathway	2	cyclic-AMP, glycolate
Melanogenesis	2	cyclic-AMP, glycolate
Prolactin signaling pathway	2	ATP, glycolate
Proximal tubule bicarbonate reclamation	2	glutamine, glycolate
Fat digestion and absorption	2	coenzyme A, glycolate
Morphine addiction	2	cyclic-AMP, glycolate
Nicotine addiction	2	4-aminobutyrate, glycolate
Pertussis	2	cyclic-AMP, glycolate

Supplementary Table 5 ^AMetabolic pathways described in the KEGG Database; ^BNumber of genes in the rat genome that are catalogued as being part of the KEGG pathway; ^CNumber of proteins detected in rat livers (at least one sample) using SWATH; ^DFraction of all rat genes in the KEGG pathway detected in rat liver using SWATH proteomics; ^EList of proteins detected from the KEGG pathway in at least one sample.

KEGG Pathway ^A	Rat Genes ^B	SWATH Proteins ^C	Coverage ^D	List of Proteins Detected ^E
Glyoxylate and dicarboxylate metabolism	28	28	1.000	Acat2, Hao1, Mut, Aco2, Hyi, Hao2, Mdh1, Shmt1, Agxt, Afmid, Mcee, Gldc, Pcca, Shmt2, Gcsh, Mdh2, Hoga1, Pccb, Grhpr, Acat1, Gldc, Dld, Pccb, Glyctk, Cs, Aco1, Amt, Glul
Lysine biosynthesis	2	2	1.000	Aadat, Aldh7a1
Citrate cycle (TCA cycle)	32	26	0.813	Idh2, Aco2, Mdh1, Pck2, Sdh, Pdhh, Pck1, Idh3a, Mdh2, Pdha1, Idh3B, Sucla2, Suclg1, Dld, Dlat, Dlst, Sdha, Acl, Suclg2, Cs, Idh3g, Aco1, Idh1, Sdhc, Ogdh, Sdhb
Propanoate metabolism	32	25	0.781	Ldha, Acat2, Mut, Acss3, Mlycd, Ehhdh, Abat, Mcee, Acaca, Dbt, Hibch, Pcca, Bckdha, Aldh6a1, Acadm, Pccb, Sucla2, Acat1, Suclg1, Dld, Pccb, Hadha, Bckdhh, Suclg2, Echsl
Valine, leucine and isoleucine degradation	56	42	0.750	Acat2, Mut, Aldh9a1, Hspa9, Hsd17b10, Aldh3a2, Acaa2, Hmgcs1, Aldh1b1, Ehhdh, Abat, Acad8, Mpv17, Mcee, Auh, Dbt, Acads, Hibch, Pcca, Hmgcs2, Aox1, Bckdha, Agxt2, Acaa1a, Aldh6a1, Acadm, Pccb, Acadsb, Mccc1, Aldh7a1, Acat1, Aox3, Dld, Pccb, Hadha, Hibadh, Mccc2, Bckdhh, Hmgcl, Ivd, Echsl, Hadhb
Fatty acid degradation	47	33	0.702	Acat2, Aldh9a1, Hspa9, Aldh3a2, Acaa2, Aldh1b1, Adh6, Ehhdh, Acox1, Acads, Cyp4a14, Acaa1a, Acsl1, Acadm, Acadsb, Aldh7a1, Acat1, Acadl, Acsl4, Eci2, Cpt1a, Hadha, Acox3, Cyp4a2, Fahd2, Acsl5, Echsl, Adh5, Cpt2, Eci1, Hadhb, Gcdh, Acadvl
D-Glutamine and D-glutamate metabolism	3	2	0.667	Gls2, Glud1
Proteasome	47	31	0.660	Psm17, Psm10, Psm13, Psm14, Psmc2, Psme1, Hadh, Psm12, Psm2, Psm1, Psma6, Psma1, Psmc1, Psm11, Psm3, Psma4, Psmc5, Psm3, Psm8, Psme2, Psmc6, Psmc4, Psma5, Psm4, Psma7, Atp6v1a, Psmc3, Psm2, Psm2, Psm1, Psm8
2-Oxocarboxylic acid metabolism	19	12	0.632	Got1, Idh2, Aco2, Aadat, Acyl1, Idh3a, Idh3B, Cs, Idh3g, Aco1, Got2, Idh1
Primary bile acid biosynthesis	16	10	0.625	Acox2, Slc27a5, Akr1d1, Cyp27a1, Baat, Akr1d1, Amacr, Hsd17b4, Cyp8b1, Scp2
Glycine, serine and threonine metabolism	40	24	0.600	Bhmt, Pipox, Gamt, Shmt1, Gcat, Agxt, Cbs, Sds, Gldc, Shmt2, Gcsh, Agxt2, Aldh7a1, Grhpr, Sardh, Gldc, Dld, Glyctk, Chdh, Maob, Amt, Pgml, Maoa, Dmgdh
Vitamin B6 metabolism	10	6	0.600	RGD1566085, Pdxk, Aox1, Pnpo, Aox3, Rbp1
Carbon metabolism	121	72	0.595	Acat2, Hao1, Got1, Cps1, Idh2, Mut, Esd, Aco2, Hao2, Aldoa, Mdh1, Idnk, Ighm, Shmt1, Aldob, Pgl, Sdh, Agxt, Ehhdh, Sds, Pdhh, Mcee, Gldc, Idh3a, Acads, Pgd, Impa1, Hibch, Pcca, Shmt2, Mdh2, Pdha1, Idh3B, Fbp1, Aldh6a1, Acadm, Pccb, Sucla2, Gapdh, Acat1, Suclg1, Pfkf, Gldc, Eno1, Pklr, Dld, Dlat, Pccb, Rgn, Dlst, Hadha, Sdha, Taldo1, Suclg2, Cs, Glud1, Idh3g, Aco1, Got2, Tpi1, Me1, Idh1, Tkt, Pgl1, Amt, Echsl, Pgml, Sdhc, Adh5, Ogdh, Tkfc, Sdhb
Butanoate metabolism	28	16	0.571	Acat2, Acsm3, Hmgcs1, Ehhdh, Abat, Bdh1, Acads, Hmgcs2, L2hgdh, Acat1, Aldh5a1, Hadha, Acsm1, Hmgcl, Echsl, Acsm5
Tryptophan metabolism	46	26	0.565	Acat2, Aldh9a1, Hspa9, Aadat, Cyp1a2, Aldh3a2, Acmsd, Cyp2c11, Aldh1b1, Haao, Ehhdh, Afmid, Ccbl2, Ccbl1, Aox1, Aldh7a1, Acat1, Aox3, Hadha, Kmo, Maob, Echsl, Kynu, Maoa, Ogdh, Gcdh
Arginine biosynthesis	20	11	0.550	Asl, Got1, Cps1, Arg1, Ass1, Glsl, Acyl1, Glud1, Otc, Got2, Glul
Pyruvate metabolism	40	22	0.550	Ldha, Acat2, Aldh9a1, Hspa9, Aldh3a2, Mdh1, Pck2, Aldh1b1, Pdhh, Pck1, Acaca, Mdh2, Pdha1, Aldh7a1, Grhpr, Acat1, Pklr, Dld, Dlat, Hagh, Me1, Glo1
Synthesis and degradation of ketone bodies	11	6	0.545	Acat2, Hmgcs1, Bdh1, Hmgcs2, Acat1, Hmgcl
Proximal tubule bicarbonate reclamation	22	12	0.545	Atp1b1, Mdh1, Pck2, Atp1b3, Nat1, Glsl, Pck1, Atp1a1, Slc25a10, Adh1, Ca2, Glud1

Supplementary Table 5 (Continued)

KEGG Pathway ^A	Rat Genes ^B	SWATH Proteins ^C	Coverage ^D	List of Proteins Detected ^E
Histidine metabolism	25	13	0.520	Ftcd, Aldh9a1, Hspa9, Aldh3a2, Cndp2, Aldh1b1, Uroc1, Amdhd1, Vars, Aldh7a1, Hal, Maob, Maoa
Ascorbate and aldarate metabolism	27	14	0.519	Aldh9a1, Hspa9, Aldh3a2, Ugt2b17, Gulo, Ugt1a1, Ugt2b37, Aldh1b1, Ugt2b15, Ugt2b10, Ugdh, Aldh7a1, Rgn, Ugt2b17
Fatty acid metabolism	54	27	0.500	Acat2, Acaa2, Pocr, Ehhadh, Acaca, Acox1, Fasn, Acads, Acaa1a, Acsl1, Acadm, Acadsb, Mecr, Acat1, Hsd17b12, Acadl, Acsl4, Cpt1a, Hadha, Oxsm, Acox3, Tecr, Acsl5, Echsl, Cpt2, Hadhb, Acadvl
Glutathione metabolism	58	29	0.500	Idh2, Gss, Gstm3, Gstk1, Ndufv3, Gpx3, Lap3, Gsr, Mgst1, Gstp1, Pgd, Impa1, Gsst1, Mgst3, Anpep, Gstm4, Gsto1, Gsst2, Gclc, Txndc12, Gclm, Gsta1, Gstm1, Gsta4, Oplah, Gpx1, Idh1, Gsta1, Gstm2
beta-Alanine metabolism	34	17	0.500	Aldh9a1, Hspa9, Aldh3a2, Cndp2, Aldh1b1, Mlycd, Ehhadh, Abat, Dpyd, Upb1, Dpys, Hi-bch, Aldh6a1, Acadm, Aldh7a1, Hadha, Echsl
Caffeine metabolism	6	3	0.500	Cyp1a2, Uox, Xdh
Pentose and glucuronate interconversions	36	17	0.472	Aldh3a2, Ugt2b17, Ugt1a1, Akr1b1, Ugt2b37, Gusb, Aldh1b1, Cryl1, Xylb, Sord, Ugt2b15, Ugt2b10, Ugdh, Ugp2, Akr1a1, Dcxr, Ugt2b17
Sulfur metabolism	13	6	0.462	Suox, Gpt, Tst, Mpst, Ethe1, Papss2
Drug metabolism - cytochrome P450	71	32	0.451	Fmo1, Cyp1a2, Ugt2b17, Ugt1a1, Gstm3, Gstk1, Ndufv3, Ugt2b37, Fmo3, Adh6, Mgst1, Fmo5, Gstp1, Ugt2b15, Ugt2b10, Aox1, Gsst1, Mgst3, Gstm4, Aox3, Gsto1, Gsst2, Cyp2e1, Gsta1, Gstm1, Gsta4, Maob, Gsta1, Gstm2, Adh5, Maoa, Ugt2b17
Pentose phosphate pathway	29	13	0.448	Aldoa, Rbks, Idnk, Ighm, Aldob, Pgl, Pgd, Impa1, Fbp1, Pfk1, Rgn, Taldo1, Tkt
One carbon pool by folate	18	8	0.444	Ftcd, Shmt1, Mthfd1, Shmt2, Aldh1l1, Aldh1l2, Atic, Amt
Nitrogen metabolism	18	8	0.444	Cps1, Ca3, Ca1, Car14, Ca5a, Ca2, Glud1, Glul
Peroxisome	88	39	0.443	Acox2, Hao1, Ech1, Ephx2, Pipox, Dhrrs4, Idh2, Baat, Hao2, Gstk1, Ndufv3, Pxmp2, Pocr, Mlycd, Phyh, Agxt, Ehhadh, Hacl1, Acox1, Slc27a2, Sars, Amacr, Acaa1a, Abcd3, Acsl1, Sod2, Hsd17b4, Acsl4, Eci2, Sod1, Acox3, Hmgcl, Scp2, Decr2, Idh1, Xdh, Acsl5, Crot, Prdx5
Biosynthesis of amino acids	83	36	0.434	Asl, Got1, Cps1, Idh2, Aco2, Aadat, Arg1, Aldoa, Shmt1, Aldob, Cbs, Ass1, Sds, Acyl1a, Idh3a, Shmt2, Idh3B, Aldh7a1, Gapdh, Pfk1, Eno1, Pklr, Pah, Taldo1, Cs, Otc, Idh3g, Aco1, Got2, Tpi1, Idh1, Tkt, Pgl1, Pgml1, Mat1a, Glul
Phenylalanine, tyrosine and tryptophan biosynthesis	7	3	0.429	Got1, Pah, Got2
Metabolism of xenobiotics by cytochrome P450	70	30	0.429	Cyp1a2, Ugt2b17, Ugt1a1, Gstm3, Ephx1, Gstk1, Ndufv3, Ugt2b37, Adh6, Mgst1, Gstp1, Ugt2b15, Ugt2b10, Gsst1, Mgst3, Gstm4, Gsto1, Gsst2, Cyp2e1, Gsta1, Gstm1, Hsd11b1, Gsta4, Akr7a2, Gsta1, Gstm2, Sult2a1, Adh5, Ugt2b17, Akr7a3
Fatty acid biosynthesis	14	6	0.429	Acaca, Fasn, Acsl1, Acsl4, Oxsm, Acsl5
Cysteine and methionine metabolism	47	20	0.426	Ldha, Got1, Bhmt, Gss, Mdh1, Cbs, Mri1, Ahcy, Sds, Bhmt2, Adi1, Mdh2, Agxt2, Tst, Mpst, Mtap, Gclc, Gclm, Got2, Mat1a
Selenocompound metabolism	19	8	0.421	Txnrd2, Scly, Ccbl2, Ccbl1, Sephs1, Txnrd1, Mars, Papss2
PPAR signaling pathway	79	33	0.418	Acox2, Fabp7, Slc27a5, Gk, Cyp27a1, Apoc3, Pck2, Fabp1, Ehhadh, Pck1, Acox1, Slc27a2, Sars, Hmgcs2, Cyp4a14, Acaa1a, Acsl1, Acadm, Acadl, Acsl4, Apoa1, Cpt1a, Acox3, Cyp8b1, Cyp4a2, Fahd2, Scp2, Dbi, Ilk, Me1, Acsl5, Cpt2, Apoa2
Chemical carcinogenesis	91	38	0.418	Cyp1a2, Cyp3a18, Ugt2b17, Ugt1a1, Gstm3, Ephx1, Gstk1, Ndufv3, Ugt2b37, Adh6, Mgst1, Cyp2c22, Ccbl2, Ccbl1, Gstp1, Ugt2b15, Ugt2b10, Cyp3a2, Gsst1, Mgst3, Gstm4, Sult1a1, Cyp2c23, Cyp2c7, Cyp2c13, Gsto1, Gsst2, Cyp2e1, Gsta1, Gstm1, Hsd11b1, Gsta4, Cyp2b3, Gsta1, Gstm2, Sult2a1, Adh5, Ugt2b17
Phenylalanine metabolism	22	9	0.409	Got1, Hpd, Pah, Glyatl1, Got2, Glyat, Maob, Mif, Maoa

(Continued)

Supplementary Table 5 (Continued)

KEGG Pathway ^A	Rat Genes ^B	SWATH Proteins ^C	Coverage ^D	List of Proteins Detected ^E
Oxidative phosphorylation	144	58	0.403	Ndufs2, Ndufv2, Ndufa6, Uqcrc2, Ndufc2, Atp6v1e1, Ndufs1, Cox7a2, Cox4i1, Ndubf4, Ndubf5, Atp6v1f, Uqcr10, Ndubf10, Cox5b, Sdhb, Ndufs8, Uqcrq, Ndufs7, Atp5f1, Ndufa8, Ndufa12, Ndufa5, Atp5j2, Atp6v0d1, Ndufa4, Atp5o, Ndufv1, Uqcrc1, Ndufa9, Atp5b, Ndubf1, Ndufs4, Atp5j, Ndufs3, Ndufa2, Atp5d, Atp5h, Ndufa10, Ndufa7, Ndubf7, Atp5i, Cox6c2, Uqcr11, Cox6a1, Sdha, Cox6b1, Atp5a1, Ndubf3, Lhpb, Atp5l, Sdhc, Ppa1, Cyc1, Uqcrfs1, Ppa2, Cox5a, Sdhb
Alanine, aspartate and glutamate metabolism	35	14	0.400	Asl, Got1, Cps1, Adsl, Agxt, Ass1, Abat, Gls2, Nit2, Agxt2, Aldh5a1, Glud1, Got2, Glul
Glycolysis/Gluconeogenesis	70	27	0.386	Ldha, Aldh9a1, Hspa9, Aldh3a2, Aldoa, Pck2, Aldh1b1, Aldob, Adh6, Pdhh, Pck1, Pdha1, Fbp1, Aldh7a1, Gapdh, Pfkf, Galm, Eno1, Pklr, Dld, Dlat, G6pc, Akr1a1, Tpi1, Pgl1, Pgml, Adh5
Tyrosine metabolism	39	15	0.385	Got1, Hpd, Adh6, Fah, Hgd, Aox1, Aox3, Gstz1, Fahd1, Got2, Maob, Mif, Adh5, Maoa, Comt
Parkinson's disease	153	58	0.379	Ndufs2, Ndufv2, Ndufa6, Uqcrc2, Ndufc2, Ndufs1, Cox7a2, Cox4i1, Ndubf4, Ndubf5, Uqcr10, Ndubf10, Cox5b, Sdhb, Ndufs8, Uqcrq, Ndufs7, Atp5f1, Ndufa8, Ndufa12, Vdac2, Ndufa5, Uba1, Htra2, Ndufa4, Prkaca, Atp5o, Ndufv1, Slc25a5, Uqcrc1, Ndufa9, Atp5b, Ndubf1, Ndufs4, Atp5j, Ndufs3, Ndufa2, Atp5d, Atp5h, Ndufa10, Ndufa7, Ndubf7, Cox6c2, Uqcr11, Cox6a1, Sdha, Vdac3, Ppif, Cox6b1, Atp5a1, Ndubf3, Vdac1, Park7, Sdhc, Cyc1, Uqcrfs1, Cox5a, Sdhb
Lysine degradation	52	19	0.365	Aass, Acat2, Pipox, Hykk, Aldh9a1, Hspa9, Aaad, Aldh3a2, Aldh1b1, Ehhadh, Phykpl, Aldh7a1, Acat1, Dlst, Hadha, Bbox1, Echs1, Ogdh, Gcdh
Retinol metabolism	83	30	0.361	Dhrs4, Cyp2a2, Cyp1a2, Cyp3a18, Ugt2b17, Ugt1a1, Ugt2b37, Aldh1a1, Adh6, Hsd17b6, Cyp2c22, Cyp2a1, Cyp4a14, Ugt2b15, Ugt2b10, Cyp3a2, Aox1, Cyp2c23, Cyp2c7, Aldh1a7, Aox3, Cyp2c13, Aldh2, Cyp4a2, Fahd2, Cyp2b3, Retsat, Adh5, Ugt2b17, Rdh3
Drug metabolism - other enzymes	56	20	0.357	Tymp, Ces1e, Ugt2b17, Ugt1a1, Ces2a, Ugt2b37, Gusb, Hprr1, Dpyd, Upb1, Dpys, Itpa, Ugt2b15, Ugt2b10, Tpmr, Umps, Xdh, Ces1d, Alb, Ugt2b17
Arginine and proline metabolism	51	18	0.353	Got1, Gamt, Aldh9a1, Hspa9, Aldh3a2, Agmat, Arg1, Cndp2, Aldh1b1, Prodh, Lap3, Prodh2, Hoga1, Aldh7a1, Got2, Maob, Oat, Maoa
Biosynthesis of unsaturated fatty acids	29	10	0.345	Baat, Acot2, Pccr, Acot4, Acox1, Acaa1a, Hsd17b12, Hadha, Acox3, Tccr
Steroid hormone biosynthesis	81	27	0.333	Akr1d1, Cyp1a2, Cyp3a18, Ugt2b17, Ugt1a1, Hsd17b8, Sult1e1, Akr1d1, Ugt2b37, Cyp2d10, Hsd17b6, Cyp2c22, Cyp2d26, Ugt2b15, Ugt2b10, Cyp3a2, Cyp2d1, Hsd17b12, Cyp2c23, Cyp2c7, Cyp2c13, Cyp2e1, Hsd11b1, Cyp2b3, Hsd3b5, Comt, Ugt2b17
Ubiquinone and other terpenoid-quinone biosynthesis	12	4	0.333	Vkorc1l1, Hpd, Coq5, Ggcs
Biotin metabolism	3	1	0.333	Oxsm
Pantothenate and CoA biosynthesis	19	6	0.316	Enpp3, Dpyd, Upb1, Dpys, Ppcc, Coasy
Fatty acid elongation	29	9	0.310	Acaa2, Acot2, Acot4, Mccr, Hsd17b12, Hadha, Tccr, Echs1, Hadhb
Alzheimer's disease	182	56	0.308	Ndufs2, Ndufv2, Ndufa6, Uqcrc2, Hsd17b10, Ndufc2, Ndufs1, Cox7a2, Cox4i1, Ndubf4, Ndubf5, Uqcr10, Ndubf10, Cox5b, Sdhb, Ndufs8, Uqcrq, Ndufs7, Atp5f1, Ndufa8, Ndufa12, Ndufa5, Ndufa4, Ide, Atp5o, Ndufv1, Uqcrc1, Apoe, Ndufa9, Atp5b, Gapdh, Ndubf1, Ndufs4, Atp5j, Ndufs3, Ndufa2, Atp5d, Calm1, Atp5h, Ndufa10, Lrp1, Ndufa7, Atp2a2, Ndubf7, Cox6c2, Uqcr11, Cox6a1, Sdha, Cox6b1, Atp5a1, Ndubf3, Sdhc, Cyc1, Uqcrfs1, Cox5a, Sdhb
Terpenoid backbone biosynthesis	23	7	0.304	Acat2, Hmgcs1, Idi1, Fdps, Hmgcs2, Acat1, Pcyox1

Supplementary Table 5 (Continued)

KEGG Pathway ^A	Rat Genes ^B	SWATH Proteins ^C	Coverage ^D	List of Proteins Detected ^E
Huntington's disease	204	62	0.304	Ndufs2, Ap2a1, Ndufv2, Ndufa6, Uqcrc2, Ndufc2, Ndufs1, Cox7a2, Cox4i1, Ndufb4, Ndufb5, Uqcr10, Ndufb10, Cox5b, Sdhc, Ndufs8, Uqcrq, Ndufs7, Atp5f1, Ndufa8, Ndufa12, Vdac2, Ndufa5, Tgm2, Ndufa4, Atp5o, Ndufv1, Slc25a5, Uqcrc1, Ap2a2, Ndufa9, Atp5b, Sod2, Ndufab1, Ndufs4, Atp5j, Ndufs3, Ndufa2, Atp5d, Ap2b1, Atp5h, Ndufa10, Cltc, Ndufa7, Ndufb7, Cox6c2, Uqcr11, Cox6a1, Sdha, Sod1, Vdac3, Ppif, Cox6b1, Atp5a1, Ndufb3, Vdac1, Gpx1, Sdhc, Cyc1, Uqcrfs1, Cox5a, Sdhb
Non-alcoholic fatty liver disease (NAFLD)	162	49	0.302	Ndufs2, Ndufv2, Ndufa6, Uqcrc2, Ndufc2, Eif2s1, Ndufs1, Cox7a2, Cox4i1, Ndufb4, Ndufb5, Uqcr10, Ndufb10, Cox5b, Rac1, Sdhc, Cdc42, Ndufs8, Uqcrq, Ndufs7, Ndufa8, Ndufa12, Ndufa5, Ndufa4, Ndufv1, Uqcrc1, Ndufa9, Ndufab1, Ndufs4, Ndufs3, Ndufa2, Pklr, Ndufa10, Ndufa7, Ndufb7, Cox6c2, Uqcr11, Cyp2e1, Cox6a1, Sdha, Cox6b1, Ndufb3, Prkag1, Sec13, Sdhc, Cyc1, Uqcrfs1, Cox5a, Sdhb
Steroid biosynthesis	20	6	0.300	Fdft1, Ebp, Dhcr7, Atad3, Nsdhl, Lipa
Protein processing in endoplasmic reticulum	168	49	0.292	Erp29, Man1a1, Ighm, Eif2s1, Rrbp1, Sec61a1, Dnajb11, Hsp90b1, Hsph1, Dnajc3, Lman2, Dnaja2, Dnaja1, Canx, Vcp, Sar1b, Pdia3, Ssr4, Hsp90aa1, Ube2d3, Pdia6, Bcap31, Hspa5, Rad23b, Hspa8, Rpn1, A1cf, Pdia4, Stt3a, Ssr1, Ddost, Prkcsh, Stt3b, Sec23a, Hsp90ab1, Ns1c, Ugg1, Txndc5, Hyou1, Sec31a, Ssr3, Sec61b, Calr, Lman1, Ubqln1, Rpn2, Ganab, P4hb, Sec24a
Cyanoamino acid metabolism	7	2	0.286	Shmt1, Shmt2
Ribosome	172	49	0.285	Rpl26, Rpl23, Rps8, Rplp1, Rplp0, Rpl35, Rpl10a, Rpl17, Rpl21, Ste2, Rps12, Rps11, Rps5, Rpl27, Rpl8, Rps4x, Rps28, Rps3, Rpl39, Rps18, Rps16, Ckb, Rps26, Rps15, Rpl10, Rpl30, Rps23, Rpl3, Rps7, Rpl32, Rpl7, Rpl18a, Rpsa, Rpl12, Rpl5, Rps9, Rpl6, Rps6, Rpl24, Rpl15, Rpl13, Rpl7a, Rpl4, Rps3a, Rpl13a, Uba52, Rpl18, Rps21, Rpl11
Starch and sucrose metabolism	53	15	0.283	Enpp3, Gys2, Ugt2b17, Ugt1a1, Pygl, Ugt2b37, Gusb, Ugt2b15, Ugt2b10, Ugdh, Ugp2, Gbe1, G6pc, Agl, Ugt2b17
Nicotinate and nicotinamide metabolism	29	8	0.276	Enpp3, Nnt, Nampt, Qprt, Aox1, Aox3, Pnp, Nadk2
Sulfur relay system	11	3	0.273	Nfs1, Tst, Mpst
Fructose and mannose metabolism	37	10	0.270	Aldoa, Akr1b1, Khk, Aldob, Sord, Fbp1, Pfkf, Gmppb, Tpi1, Gmppa
Linoleic acid metabolism	41	11	0.268	Cyp1a2, Cyp3a18, Pla2g12b, Cyp4f1, Cyp2c22, Cyp3a2, Cyp2c23, Cyp2c7, Cyp2c13, Cyp2e1, Cyp2j3
Galactose metabolism	32	8	0.250	Glb1, Akr1b1, Gale, Galk1, Pfkf, Ugp2, Galm, G6pc
Amino sugar and nucleotide sugar metabolism	48	12	0.250	Hexa, Gne, Pgm3, Cyb5r3, Gale, Galk1, Ugdh, Nans, Ugp2, Gnpnat1, Gmppb, Gmppa
Riboavin metabolism	4	1	0.250	Blvrb
Valine, leucine and isoleucine biosynthesis	4	1	0.250	Sds
Cardiac muscle contraction	81	20	0.247	Uqcrc2, Cox7a2, Atp1b1, Cox4i1, Tpm3, Uqcr10, Atp1b3, Cox5b, Uqcrq, Tpm1, Uqcrc1, Atp1a1, Atp2a2, Cox6c2, Uqcr11, Cox6a1, Cox6b1, Cyc1, Uqcrfs1, Cox5a
Aminoacyl-tRNA biosynthesis	69	16	0.232	Rars, Qars, Hars, Farsb, Iars2, Yars, Dars, Tars, Cars, Nars, Aars, Eprs, Farsa, Mars, Kars, Gars
Protein export	26	6	0.231	Sec61a1, Hspa5, Srp72, Spcs3, Sec11a, Sec61b
Fat digestion and absorption	40	9	0.225	Acat2, Pla2g12b, Cyp4f1, Fabp1, Apob, Apoa4, Mttp, Apoa1, Got2
Arachidonic acid metabolism	81	18	0.222	Ephx2, Pla2g12b, Cyp4f1, Ptges3, Gpx3, Cyp2c22, Cyp4a14, Lta4h, Cyp2c23, Cyp2c7, Cyp2c13, Cyp2e1, Cyp4a2, Fahd2, Cyp2b3, Gpx1, Cyp2j3, Ptges2
Bacterial invasion of epithelial cells	81	17	0.210	Dnm2, Rac1, Arpc3, Cdc42, Rhoa, Itgb1, Fn1, Arpc5, Actb, Arpc4, Vcl, Ctnna1, H6pd, Ctnnb1, Arpc2, Cltc, Ilk
Bile secretion	72	15	0.208	Slc27a5, Baat, Abcc2, Atp1b1, Abcb11, Slco1a4, Ndufs5, Ephx1, Atp1b3, Slc22a7, Prkaca, Ugt2b15, Atp1a1, Ca2, Sult2a1

(Continued)

Supplementary Table 5 (Continued)

KEGG Pathway ^A	Rat Genes ^B	SWATH Proteins ^C	Coverage ^D	List of Proteins Detected ^E
Thyroid hormone synthesis	69	14	0.203	Iyd, Atp1b1, Hsp90b1, Gpx3, Atp1b3, Gsr, Canx, Prkaca, Hspa5, Asgr2, Atp1a1, Pdia4, Asgr1, Gpx1
Thiamine metabolism	5	1	0.200	Nfs1
alpha-Linolenic acid metabolism	25	5	0.200	Pla2g12b, Cyp4f1, Acox1, Acaa1a, Acox3
Glycosaminoglycan degradation	21	4	0.190	Glb1, Hexa, Gusb, Naglu
Collecting duct acid secretion	27	5	0.185	Atp6v1e1, Atp6v1f, Atp6v0d1, Ca2, Slc4a1
Endocrine and other factor-regulated calcium reabsorption	49	9	0.184	Ap2a1, Atp1b1, Dnm2, Atp1b3, Prkaca, Ap2a2, Atp1a1, Ap2b1, Cltc
Glycerolipid metabolism	60	11	0.183	Gk, Aldh9a1, Hspa9, Aldh3a2, Mgll, Akr1b1, Aldh1b1, Aldh7a1, Glyctk, Akr1a1, Tkfc
Taurine and hypotaurine metabolism	11	2	0.182	Baat, Csad
N-Glycan biosynthesis	50	9	0.180	Man1a1, Ighm, Rpn1, A1cf, Stt3a, Ddost, Stt3b, Rpn2, Ganab
Glucagon signaling pathway	102	18	0.176	Ldha, Gys2, Pygl, Pck2, Pdhb, Pck1, Acaca, Prkaca, Pdha1, Fbp1, Pfk1, Calm1, Cpt1a, G6pc, Prkag1, Sec13, Pgam1, Slc2a2
Adherens junction	74	12	0.162	Actn1, Actn4, Rac1, Cdc42, Rhoa, Actb, Ctnnd1, Acp1, Vcl, Ctnna1, H6pd, Ctnnb1
Arrhythmogenic right ventricular cardiomyopathy (ARVC)	74	12	0.162	Actn1, Actn4, Itgb1, Jup, Actb, Itgb3, Ctnna1, H6pd, Ctnnb1, Atp2a2, Lmna, Itga1
Complement and coagulation cascades	82	13	0.159	Map1, Fgg, Mbl2, Serpina1, C4a, Serpina1, Fga, Serpinc1, Fgb, Vtn, Serpinf2, Plg, Fh
Carbohydrate digestion and absorption	44	7	0.159	Slc37a4, Atp1b1, Atp1b3, Atp1a1, G6pc, Slc2a2, Aldh4a1
Other glycan degradation	19	3	0.158	Glb1, Hexa, Man2c1
Lysosome	129	20	0.155	Glb1, Hexa, Scarb2, Tpp1, Ctsc, Psap, Ctsh, Gusb, Ctsb, Asah1, Lamp2, Atp6v0d1, Naga, Naglu, Ctca, Ap1b1, Cltc, Gm2a, Lipa, Ctcd
AMPK signaling pathway	129	20	0.155	Gys2, Rab11b, Pck2, Mlycd, Pck1, Acaca, Fasn, Rab10, Cyp2c6, Cab39, Rab2a, Fbp1, Rab14, Eef2, Pfk1, Ppp2r1a, Cpt1a, G6pc, Prkag1, Sec13
Folate biosynthesis	13	2	0.154	Spr, Qdpr
RNA transport	164	25	0.152	Eif4a2, Eif3c, Eif2s1, Eif3d, Eif2s3y, Eif3b, Eif3a, Eif5, Xpo1, Eif4g1, Sumo3, Rangap1, Pabpc1, Eif3g, Kpnb1, Eif3j, Eif3i, Ddx39b, Eif3f, Eif4a1, Upf1, Eif4a3, Ran, Eef1a1, Cyfip1
Synaptic vesicle cycle	62	9	0.145	Ap2a1, Atp6v1e1, Dnm2, Atp6v1f, Atp6v0d1, Ap2a2, Ap2b1, Cltc, Napa
Vasopressin-regulated water reabsorption	43	6	0.140	Rab5c, Rab11b, Rab5b, Prkaca, Arhgdia, Dync1h1
Central carbon metabolism in cancer	65	9	0.138	Ldha, Gls2, Pdhb, Pdha1, Sirt3, Isoc2, Pfk1, Pgam1, Slc2a2
Insulin resistance	111	15	0.135	Slc27a5, Gys2, Pygl, Pck2, Ppp2r4, Pck1, Slc27a2, Sars, Ppp1ca, Cpt1a, G6pc, Stat3, Prkag1, Sec13, Slc2a2
Glycosphingolipid biosynthesis - ganglio series	15	2	0.133	Glb1, Hexa
Glycosphingolipid biosynthesis - globo series	15	2	0.133	Hexa, Naga
Leukocyte transendothelial migration	120	16	0.133	Actn1, Msn, Actn4, Rac1, Cdc42, Rhoa, Itgb1, F11r, Actb, Myl12b, Ctnnd1, Vcl, Ctnna1, H6pd, Ctnnb1, Rap1b
Purine metabolism	181	24	0.133	Enpp3, Nme2, Adk, Adsl, Nme1, RGD1309350, Uox, Hpirt1, Gda, Itpa, Ak3, Paics, Nme3, Entpd5, Dguok, Atic, Pklr, Pnp, Aprt, Ak2, Papss2, Xdh, Urad, Ak4
Adipocytokine signaling pathway	75	10	0.133	Pck2, Pck1, Acsl1, Acsl4, Cpt1a, G6pc, Stat3, Prkag1, Sec13, Acsl5
Antigen processing and presentation	98	13	0.133	Ctsb, Psme1, Hadh, Hspa4, Canx, Pdia3, Hsp90aa1, Hspa5, Hspa8, RT1-A2, Psme2, Hsp90ab1, Calr

Supplementary Table 5 (Continued)

KEGG Pathway ^A	Rat Genes ^B	SWATH Proteins ^C	Coverage ^D	List of Proteins Detected ^E
Salmonella infection	83	11	0.133	Rac1, Arpc3, Cdc42, Pfn1, Flnb, Arpc5, Actb, Arpc4, Arpc2, Rab7a, Dync1h1
Pyrimidine metabolism	106	14	0.132	Tymp, Txnrd2, Nme2, Nme1, Dpyd, Upb1, Dpys, Itpa, Nme3, Entpd5, Umps, Txnrd1, Pnp, Cmpk1
Phagosome	198	26	0.131	Tfrc, Mbl2, Atp6v1e1, Tubb4b, Vamp3, Sec61a1, Rab5c, Atp6v1f, Rab5b, Rac1, Tuba4a, Canx, Lamp2, Itgb1, Atp6v0d1, Sec22b, Actb, Itgb3, RT1-A2, Rab7a, Dync1h1, Sec61b, Calr, Tubb5, Dhfr, Tubb2a
ABC transporters	47	6	0.128	Abcc2, Abca6, Abcb11, Abcd3, Abcc6, Abca8a
Epstein-Barr virus infection	229	29	0.127	Vim, Ywhab, Psmc7, Snd1, Psmc13, Psmc14, Psmc2, Xpo1, Psmc12, Psmc1, Prkaca, Psmc1, Psmc11, Hspa8, Ywhag, Ywhaz, Psmc3, Psmc5, Psmc8, RT1-A2, Ywhah, Stat3, Psmc6, Psmc4, Ddx58, Ran, Ywhae, Psmc3, Psmc2
Renin-angiotensin system	32	4	0.125	Prep, Anpep, Ctsa, Nln
Vitamin digestion and absorption	24	3	0.125	Apob, Apoa4, Apoa1
Endocytosis	288	35	0.122	Tfrc, Ap2a1, Snx2, Snx5, Dnm2, Arf6, Rab5c, Rab11b, Rab5b, Arpc3, Cdc42, Vps26b, Vps4b, Capza2, Vps25, Rhoa, Pdc6ip, Rab10, Cyp2c6, Capza1, Arpc5, Arf3, Ap2a2, Hspa8, Arpc4, Ap2b1, Arpc2, Cltc, RT1-A2, Rab7a, Vps35, Arfgap2, Vps29, Ehd3, Capzb
Legionellosis	58	7	0.121	Eef1g, Ggct, Vcp, Sar1b, Sec22b, Hspa8, Eef1a1
HIF-1 signaling pathway	109	13	0.119	Tfrc, Ldha, Tceb2, Aldoa, Pdhh, Cul2, Pdha1, Gapdh, Pfk1, Eno1, Stat3, Rps6, Pgk1
Hypertrophic cardiomyopathy (HCM)	85	10	0.118	Tpm3, Itgb1, Tpm1, Actb, Itgb3, Atp2a2, Lmna, Prkag1, Sec13, Itga1
Prion diseases	34	4	0.118	Prkaca, Hspa5, Stip1, Sod1
Ether lipid metabolism	44	5	0.114	Tmem86b, Pla2g12b, Cyp4f1, Cept1, Pafah1b2
Insulin signaling pathway	140	16	0.114	Gys2, Pygl, Pck2, Pck1, Acaca, Fasn, Prkaca, Fbp1, Calm1, Pklr, Ppp1ca, G6pc, Prkar2a, Rps6, Prkag1, Sec13
Regulation of actin cytoskeleton	221	25	0.113	Actn1, Msn, Actn4, Rac1, Arpc3, Cdc42, Rhoa, Itgb1, Iqgap2, Pfn1, C2, Diaph1, Fn1, Arpc5, Actb, Arpc4, Myl12b, Itgb3, Vcl, Ppp1ca, Arpc2, Rdx, Itga1, Cyfip1, Cfl1
Platelet activation	127	14	0.110	Fgg, Fga, Rhoa, Itgb1, Prkaca, Stim1, Pblid, Fgb, Actb, Myl12b, Itgb3, Ppp1ca, Tln1, Rap1b
Fc gamma R-mediated phagocytosis	91	10	0.110	Dnm2, Arf6, Rac1, Arpc3, Cdc42, Cfl2, Arpc5, Arpc4, Arpc2, Cfl1
Mineral absorption	46	5	0.109	Fth1, Atp1b1, Atp1b3, Atp1a1, Atox1
Spliceosome	137	15	0.109	Snrpd3, Snrpd1, Hnrnpa1, Ddx5, Hnrnpk, U2af2, Hspa8, Ddx39b, Snrpa1, Srsf1, Hnrnpa3, Hnrnpu, Hnrnpm, Eif4a3, Tra2b
mRNA surveillance pathway	93	10	0.108	Gspt1, Cpsf6, Dazap1, Etf1, Pabpc1, Ddx39b, Ppp2r1a, Ppp1ca, Upf1, Eif4a3
Focal adhesion	206	22	0.107	Actn1, Col6a1, Actn4, Rac1, Cdc42, Rhoa, Itgb1, Flnb, Diaph1, Fn1, Actb, Myl12b, Vtn, Itgb3, Parva, Vcl, Ctnnb1, Ppp1ca, Tln1, Ilk, Rap1b, Itga1
Apoptosis	141	15	0.106	Ctsc, Eif2s1, Casp6, Ctsh, Ctsb, Tuba4a, Htra2, Actb, Parp3, Sptan1, Lmnbl, Endog, Lmna, Aifm1, Ctscd
Vascular smooth muscle contraction	123	13	0.106	Pla2g12b, Cyp4f1, Myh11, Rhoa, Prkaca, Cyp4a14, Acta2, Calm1, Ppp1ca, Cyp4a2, Fahd2, Myl6, Cald1
Tight junction	143	15	0.105	Actn1, Actn4, Myh11, Cdc42, Rhoa, F11r, Actb, Myl12b, Ctnna1, H6pd, Sptan1, Vapa, Ppp2r1a, Ctnnb1, Myh9
Pancreatic secretion	97	10	0.103	Pla2g12b, Cyp4f1, Atp1b1, Atp1b3, Rac1, Rhoa, Atp1a1, Atp2a2, Ca2, Rap1b
Thyroid cancer	29	3	0.103	Tpm3, Tfg, Ctnnb1
Dilated cardiomyopathy	91	9	0.099	Tpm3, Itgb1, Tpm1, Prkaca, Actb, Itgb3, Atp2a2, Lmna, Itga1

(Continued)

Supplementary Table 5 (Continued)

KEGG Pathway ^A	Rat Genes ^B	SWATH Proteins ^C	Coverage ^D	List of Proteins Detected ^E
Viral carcinogenesis	235	23	0.098	Actn1, Ddx3x, Actn4, Ywhab, Snd1, Rac1, Cdc42, Ddb1, Rhoa, Hnrnpk, Atp6v0d1, Prkaca, Hist1h2bd, Psmc1, Dnaja3, Ywhag, Ywhaz, Hist1h4b, RT1-A2, Ywhah, Stat3, Vdac3, Ywhae
Pertussis	73	7	0.096	C4a, Serping1, Rhoa, Itgb1, Cfl2, Calm1, Cfl1
Serotonergic synapse	125	12	0.096	Cyp2d10, Cyp2c22, Cyp2d26, Prkaca, Cyp2d1, Gnb2, Cyp2c23, Cyp2c7, Cyp2c13, Maob, Cyp2j3, Maoa
Inflammatory mediator regulation of TRP channels	115	11	0.096	Cyp2c22, Prkaca, Cyp4a14, Cyp2c23, Cyp2c7, Calm1, Cyp2c13, Ppp1ca, Cyp4a2, Fahd2, Cyp2j3
Gastric acid secretion	74	7	0.095	Atp1b1, Atp1b3, Prkaca, Actb, Atp1a1, Calm1, Ca2
Proteoglycans in cancer	203	19	0.094	Msn, Rac1, Cdc42, Ddx5, Rhoa, Itgb1, Prkaca, Flnb, Fn1, F2, Lum, Actb, Vtn, Itgb3, Ctnnb1, Ppp1ca, Stat3, Rps6, Rdx
Longevity regulating pathway – multiple species	65	6	0.092	Prkaca, Hspa8, Sod2, Sod1, Prkag1, Sec13
Staphylococcus aureus infection	57	5	0.088	Fgg, Mbl2, C4a, Plg, Fh
Amoebiasis	106	9	0.085	Actn1, Actn4, Arg1, Rab5c, Rab5b, Prkaca, Fn1, Vcl, Rab7a
Systemic lupus erythematosus	135	11	0.081	Actn1, Snrpd3, Actn4, Snrpd1, C4a, H2afv, H2afy, Hist1h2bd, Hist1h4b, H2afx, Ssb
Oocyte meiosis	113	9	0.080	Ywhab, Prkaca, Ywhag, Ywhaz, Calm1, Ppp2r1a, Ppp1ca, Ywhah, Ywhae
GABAergic synapse	90	7	0.078	Gphn, Nat1, Abat, Glis2, Prkaca, Gnb2, Glul
Amphetamine addiction	65	5	0.077	Prkaca, Calm1, Ppp1ca, Maob, Maoa
Endometrial cancer	52	4	0.077	Ctnna1, H6pd, Ctnnb1, Ilk
Thyroid hormone signaling pathway	119	9	0.076	Atp1b1, Atp1b3, Prkaca, Actb, Stat1, Atp1a1, Itgb3, Ctnnb1, Atp2a2
African trypanosomiasis	40	3	0.075	Hba-a1, Apoa1, Hbb
Renal cell carcinoma	68	5	0.074	Tceb2, Rac1, Cdc42, Cul2, Rap1b
Maturity onset diabetes of the young	27	2	0.074	Pklr, Slc2a2
Aldosterone-regulated sodium reabsorption	41	3	0.073	Atp1b1, Atp1b3, Atp1a1
Estrogen signaling pathway	96	7	0.073	Hsp90b1, Hsp90aa1, Prkaca, Hspa8, Calm1, Hsp90ab1, Fkbp4
ECM-receptor interaction	84	6	0.071	Col6a1, Itgb1, Fn1, Vtn, Itgb3, Itga1
Hippo signaling pathway	156	11	0.071	Ywhab, Actb, Ywhag, Ywhaz, Ctnna1, H6pd, Ppp2r1a, Ctnnb1, Ppp1ca, Ywhah, Ywhae
Amyotrophic lateral sclerosis (ALS)	56	4	0.071	Rac1, Tomm40, Sod1, Gpx1
Influenza A	171	12	0.070	Eif2s1, Dnajc3, Xpo1, Fdps, Actb, Stat1, Hspa8, Stat2, Ddx39b, Plg, Ddx58, Vdac1
cGMP-PKG signaling pathway	171	12	0.070	Atp1b1, Atp1b3, Rhoa, Vdac2, Slc25a5, Atp1a1, Calm1, Ppp1ca, Atp2a2, Vdac3, Ppif, Vdac1
Ovarian steroidogenesis	57	4	0.070	Acot2, Prkaca, Hsd3b5, Cyp2j3
Gap junction	88	6	0.068	Tubb4b, Tuba4a, Prkaca, Tubb5, Dhfr, Tubb2a
Adrenergic signaling in cardiomyocytes	148	10	0.068	Atp1b1, Tpm3, Atp1b3, Tpm1, Prkaca, Atp1a1, Calm1, Ppp2r1a, Ppp1ca, Atp2a2
Circadian rhythm	30	2	0.067	Prkag1, Sec13
Salivary secretion	76	5	0.066	Atp1b1, Atp1b3, Prkaca, Atp1a1, Calm1
Hepatitis B	139	9	0.065	Ddx3x, Ywhab, Ddb1, Stat1, Stat2, Ywhaz, Stat3, Vdac3, Ddx58
PI3K-Akt signaling pathway	336	22	0.065	Col6a1, Ywhab, Gys2, Hsp90b1, Pck2, Rac1, Itgb1, Pck1, Hsp90aa1, Fn1, Gnb2, Ywhag, Ywhaz, Vtn, Itgb3, Ppp2r1a, G6pc, Ywhah, Rps6, Hsp90ab1, Ywhae, Itga1
Cocaine addiction	46	3	0.065	Prkaca, Maob, Maoa
Glycerophospholipid metabolism	95	6	0.063	Gpd1, Pla2g12b, Cyp4f1, Lypla1, Cept1, Pcyt2
Pancreatic cancer	65	4	0.062	Rac1, Cdc42, Stat1, Stat3
Long-term potentiation	65	4	0.062	Prkaca, Calm1, Ppp1ca, Rap1b

Supplementary Table 5 (Continued)

KEGG Pathway ^A	Rat Genes ^B	SWATH Proteins ^C	Coverage ^D	List of Proteins Detected ^E
Dopaminergic synapse	130	8	0.062	Prkaca, Gnb2, Calm1, Ppp2r1a, Ppp1ca, Maob, Maoa, Comt
SNARE interactions in vesicular transport	33	2	0.061	Vamp3, Sec22b
cAMP signaling pathway	196	12	0.061	Atp1b1, Atp1b3, Rac1, Rhoa, Acox1, Prkaca, Atp1a1, Calm1, Ppp1ca, Atp2a2, Acox3, Rap1b
Alcoholism	179	11	0.061	H2afv, Prkaca, H2afy, Hist1h2bd, Gnb2, Calm1, Ppp1ca, Hist1h4b, Maob, H2afx, Maoa
Measles	138	8	0.058	Msn, Eif2s1, Stat1, Hspa8, Stat2, Stat3, Ddx58, Gnb2l1
Insulin secretion	86	5	0.058	Atp1b1, Atp1b3, Prkaca, Atp1a1, Slc2a2
AGE-RAGE signaling pathway in diabetic complications	104	6	0.058	Rac1, Cdc42, Diaph1, Fn1, Stat1, Stat3
Toxoplasmosis	123	7	0.057	Irgm, Igtp, Itgb1, Stat1, Hspa8, Stat3, Ppif
MicroRNAs in cancer	143	8	0.056	Vim, Gls2, Rhoa, Hnrnpk, Tpm1, Itgb3, Stat3, Rdx
Prostate cancer	89	5	0.056	Hsp90b1, Hsp90aa1, Gstp1, Ctnnb1, Hsp90ab1
Rap1 signaling pathway	216	12	0.056	Rac1, Cdc42, Rhoa, Itgb1, Pfn1, Actb, Ctnnd1, Itgb3, Calm1, Ctnnb1, Tln1, Rap1b
Neurotrophin signaling pathway	126	7	0.056	Rac1, Cdc42, Rhoa, Arhgdia, Calm1, Rap1b, Ywhae
Oxytocin signaling pathway	160	9	0.056	Rhoa, Prkaca, Actb, Eef2, Calm1, Ppp1ca, Prkag1, Sec13, Myl6
Protein digestion and absorption	93	5	0.054	Col6a1, Atp1b1, Atp1b3, Dpp4, Atp1a1
Pathways in cancer	400	21	0.053	Tceb2, Tpm3, Hsp90b1, Rac1, Cdc42, Rhoa, Itgb1, Cul2, Hsp90aa1, Prkaca, Jup, Fn1, Tfg, Stat1, Ctlp1, Gnb2, Ctnna1, H6pd, Ctnnb1, Stat3, Hsp90ab1
Calcium signaling pathway	187	10	0.053	Vdac2, Prkaca, Slc25a5, Stim1, Pblid, Calm1, Atp2a2, Vdac3, Ppif, Vdac1
Ras signaling pathway	232	12	0.052	Pla2g12b, Cyp4f1, Arf6, Rab5c, Rab5b, Rac1, Cdc42, Rhoa, Prkaca, Gnb2, Calm1, Rap1b
NOD-like receptor signaling pathway	58	3	0.052	Hsp90b1, Hsp90aa1, Hsp90ab1
Malaria	59	3	0.051	Hba-a1, Hbb, Lrp1
Herpes simplex infection	217	11	0.051	Eif2s1, Eef1d, Hnrnpk, Stat1, Stat2, Ppp1ca, Srsf1, RT1-A2, Ddx58, Taf3, C1qbp
FoxO signaling pathway	136	7	0.051	Pck2, Pck1, Sod2, G6pc, Stat3, Prkag1, Sec13
Chemokine signaling pathway	178	9	0.051	Rac1, Cdc42, Rhoa, Prkaca, Stat1, Gnb2, Stat2, Stat3, Rap1b
Ubiquitin mediated proteolysis	141	7	0.050	Tceb2, Ube2n, Uba6, Ddb1, Cul2, Uba1, Ube2d3
Tuberculosis	184	9	0.049	Rab5c, Rab5b, Rhoa, Lamp2, Atp6v0d1, Stat1, Calm1, Rab7a, Ctsc
RNA degradation	82	4	0.049	Pabpc1, Pfk1, Eno1, Wdr61
Hematopoietic cell lineage	86	4	0.047	Tfrc, Anpep, Itgb3, Itga1
RIG-I-like receptor signaling pathway	64	3	0.047	Ddx3x, Ddx58, Tkfc
Hepatitis C	129	6	0.047	Eif2s1, Stat1, Stat2, Ppp2r1a, Stat3, Ddx58
Colorectal cancer	64	3	0.047	Rac1, Rhoa, Ctnnb1
Axon guidance	130	6	0.046	Rac1, Cdc42, Rhoa, Itgb1, Cfl2, Cfl1
Renin secretion	67	3	0.045	Ctsb, Prkaca, Calm1
HTLV-I infection	293	13	0.044	Canx, Xpo1, Vdac2, Fdps, Prkaca, Slc25a5, Ctnnb1, RT1-A2, Vdac3, Vdac1, Tln1, Ran, Calr
Viral myocarditis	93	4	0.043	Rac1, Eif4g1, Actb, RT1-A2
Glutamatergic synapse	115	5	0.043	Nat1, Gls2, Prkaca, Gnb2, Glul
Nucleotide excision repair	47	2	0.043	Ddb1, Rad23b
Longevity regulating pathway - mammal	94	4	0.043	Prkaca, Sod2, Prkag1, Sec13
Mismatch repair	23	1	0.043	Ssbp1

(Continued)

Supplementary Table 5 (Continued)

KEGG Pathway ^A	Rat Genes ^B	SWATH Proteins ^C	Coverage ^D	List of Proteins Detected ^E
Sphingolipid metabolism	49	2	0.041	Glb1, Asah1
Sphingolipid signaling pathway	124	5	0.040	Rac1, Asah1, Rhoa, Ppp2r1a, Ctsd
Phototransduction	25	1	0.040	Calm1
Cell cycle	127	5	0.039	Ywhab, Ywhag, Ywhaz, Ywhah, Ywhae
Retrograde endocannabinoid signaling	103	4	0.039	Mgll, Prkaca, Gnb2, Faah
Prolactin signaling pathway	76	3	0.039	Stat1, Stat3, Slc2a2
Type II diabetes mellitus	52	2	0.038	Pklr, Slc2a2
Aldosterone synthesis and secretion	84	3	0.036	Prkaca, Calm1, Hsd3b5
Homologous recombination	28	1	0.036	Ssbp1
Acute myeloid leukemia	57	2	0.035	Jup, Stat3
Regulation of lipolysis in adipocytes	59	2	0.034	Mgll, Prkaca
Wnt signaling pathway	145	5	0.034	Rac1, Rhoa, Prkaca, Ctbp1, Ctnnb1
Rheumatoid arthritis	92	3	0.033	Atp6v1e1, Atp6v1f, Atp6v0d1
VEGF signaling pathway	60	2	0.033	Rac1, Cdc42
GnRH signaling pathway	92	3	0.033	Cdc42, Prkaca, Calm1
Progesterone-mediated oocyte maturation	90	3	0.033	Hsp90aa1, Prkaca, Hsp90ab1
Jak-STAT signaling pathway	151	5	0.033	Aox1, Stat1, Stat2, Aox3, Stat3
MAPK signaling pathway	259	8	0.031	Rac1, Cdc42, Prkaca, Ppm1b, Flnb, Ecsit, Hspa8, Rap1b
Circadian entrainment	97	3	0.031	Prkaca, Gnb2, Calm1
mTOR signaling pathway	64	2	0.031	Cab39, Rps6
Inflammatory bowel disease (IBD)	65	2	0.031	Stat1, Stat3
Osteoclast differentiation	134	4	0.030	Rac1, Stat1, Stat2, Itgb3
Melanogenesis	99	3	0.030	Prkaca, Calm1, Ctnnb1
DNA replication	36	1	0.028	Ssbp1
Leishmaniasis	75	2	0.027	Itgb1, Stat1
Inositol phosphate metabolism	74	2	0.027	Aldh6a1, Tpi1
Base excision repair	37	1	0.027	Parp3
Bladder cancer	40	1	0.025	Tymp
TGF-beta signaling pathway	86	2	0.023	Rhoa, Ppp2r1a
Ribosome biogenesis in eukaryotes	88	2	0.023	Xpo1, Ran
Small cell lung cancer	88	2	0.023	Itgb1, Fn1
Taste transduction	89	2	0.022	Prkaca, Aldh4a1
Morphine addiction	92	2	0.022	Prkaca, Gnb2
Basal transcription factors	45	1	0.022	Taf3
Toll-like receptor signaling pathway	97	2	0.021	Rac1, Stat1
Hedgehog signaling pathway	47	1	0.021	Prkaca
Phospholipase D signaling pathway	148	3	0.020	Dnm2, Arf6, Rhoa
Choline metabolism in cancer	101	2	0.020	Lypla1, Rac1
Intestinal immune network for IgA production	49	1	0.020	Pigr
Notch signaling pathway	52	1	0.019	Ctbp1
Chagas disease (American trypanosomiasis)	107	2	0.019	Ppp2r1a, Calr
T cell receptor signaling pathway	109	2	0.018	Cdc42, Rhoa
Cholinergic synapse	112	2	0.018	Prkaca, Gnb2
TNF signaling pathway	110	2	0.018	Dnm1l, Hpx

Supplementary Table 5 (Continued)

KEGG Pathway ^A	Rat Genes ^B	SWATH Proteins ^C	Coverage ^D	List of Proteins Detected ^E
Basal cell carcinoma	55	1	0.018	Ctnnb1
Cytosolic DNA-sensing pathway	56	1	0.018	Ddx58
Transcriptional misregulation in cancer	174	3	0.017	Fus, Ddx5, Jup
Cell adhesion molecules (CAMs)	172	3	0.017	Itgb1, F11r, RT1-A2
Long-term depression	62	1	0.016	Ppp2r1a
Glioma	65	1	0.015	Calm1
Graft-versus-host disease	68	1	0.015	RT1-A2
B cell receptor signaling pathway	74	1	0.014	Rac1
Fc epsilon RI signaling pathway	70	1	0.014	Rac1
Signaling pathways regulating pluripotency of stem cells	142	2	0.014	Ctnnb1, Stat3
Allograft rejection	72	1	0.014	RT1-A2
Chronic myeloid leukemia	76	1	0.013	Ctbp1
Type I diabetes mellitus	77	1	0.013	RT1-A2
Autoimmune thyroid disease	79	1	0.013	RT1-A2
NF-kappa B signaling pathway	95	1	0.011	Ddx58
Natural killer cell mediated cytotoxicity	101	1	0.010	Rac1
Phosphatidylinositol signaling system	96	1	0.010	Calm1
Neuroactive ligand-receptor interaction	292	1	0.003	Plg
Olfactory transduction	1249	2	0.002	Prkaca, Calm1

Statistically Significant Proteins (SSPs)		Statistically Significant Metabolites (SSMs)	
Reaction	Gene	Metabolite ID	Metabolite Name
R2859	Cyp2a2, Cyp1a2	nadph[r]	Nicotinamide adenine dinucleotide phosphate - reduced
R2886	Cyp2a2, Cyp1a2	frdp[r]	Farnesyl diphosphate
R3423	Fdft1		
R3972	Fdft1		
R6372	Cyp2a2, Cyp1a2		
R6658	Fmo1		
R6687	Cyp2a2, Cyp1a2

Supplementary Figure 1 The benefit of our algorithm was to identify co-factor driven interactions. In this module, we observed a significant up-regulation of the first specific enzyme of cholesterol biosynthesis, Farnesyl-Diphosphate Farnesyltransferase 1. Cholesterol biosynthesis is driven by the cofactor NADPH, which we also observe a significant upregulation of.

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Statistically Significant Proteins (SSPs)		Statistically Significant Metabolites (SSMs)	
Reaction	Gene	Metabolite ID	Metabolite Name
R1772	Ftcd	nadph[c]	Nicotinamide adenine dinucleotide phosphate - reduced
R2080	Ftcd		
R2329	Aldh9a1,	coa[c]	Coenzyme A
	Aldh3a2	atp[c]	ATP
R2602	Mthfd1	adp[c]	ADP
R2603	Mthfd1	glu-L[c]	L-glutamate(1-)
R6367	Cyp2a2,	dhf[c]	7,8-Dihydrofolate
	Cyp1a2	im4ac[c]	imidazol-4-ylacetate
R6406	Aldh9a1,		
	Aldh3a2		
R6408	Aldh9a1,		
	Aldh3a2

Supplementary Figure 2 It has been well documented in human and rodent models of RYGB like that folate deficiency occurs after RYGB⁵⁸⁰. Here we observe an upregulation of Formyltetrahydrofolate Synthetase 1(MTHFD1) cofactors and a down-regulation of the protein itself suggesting this may be a key protein involved in the observed phenotype of folate deficiency after RYGB.

Supplementary Figure 3 Here, we observed a significant increase of cofactor driven production of Nucleoside diphosphate kinase 2 (Nme2) which is directly involved in pyrimidine metabolism. Previous work from our lab has shown increased production of purine and pyrimidine metabolites from the small intestine after RYGB surgery¹⁰.

Statistically Significant Proteins (SSPs)		Statistically Significant Metabolites (SSMs)	
Reaction	Gene	Metabolite ID	Metabolite Name
R84	Got1	glu_L[c]	L-glutamate(1-)
R240	Aco2	glu_L[m]	L-glutamate(1-)
R2313	Idh2	4abut[m]	4-Aminobutanoate
R2365	Aadat	atp[c]	ATP
R2380	Got1	adp[c]	ADP
R3904	Idh2	nadph[m]	Nicotinamide adenine dinucleotide phosphate - reduced
R3922	Aadat

Supplementary Figure 4 The vast majority of metabolites and proteins of the TCA cycle are up-regulated. Interestingly, isocitrate dehydrogenase II, which is responsible for catalyzing the production of alpha ketoglutarate from 2-oxoglutarate is significantly upregulated. As TCA cycle metabolism is increased, this may lead to a significant increase in ATP generation potentially contributing to the accelerated metabolic phenotype observed after RYGB. Furthermore results indicate a significant upregulation of glutamate, which is an essential excitatory neurotransmitter which may be responsible for changes we observed in neuronal signaling pathways.

Supplementary Figure 5 Key proteins involved in lipid metabolism such as ACAT2 are upregulated. As recent literature has demonstrated significant changes in bile acid metabolism after RYGB, our findings follow suit as we observed a significant increase in key bile acid proteins such as BAAT, which is directly involved in the formation of bile acid-amino acid conjugates. An increase in bile acids throughout the gastrointestinal tract may lead to enhanced lipid and fat-soluble vitamin absorption.

Supplementary Figure 6 The distribution of the maximum module shortest path distance between two nodes for randomly generated sub-networks of size 20.

Supplementary Figure 7 Based on the module presented in **Fig. 4a** highlighting bile acid metabolism, we found a network-based relationship between Baat, Slc27a5, and Enpp3. We tested how much run-time would be required to identify a module encompassing all three of these enzymes at various module sizes using the seed reaction (Rs) as Baat.

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