

## SUPPLEMENTARY ONLINE MATERIAL

### FULL METHODS ONLINE

#### **Social Preference**

Social preference was tested using a three-chambered box similar to what has been previously described<sup>1</sup>. Briefly, a Plexiglas box (60cm L x 60cm W x 30cm H) was divided into 3 equal compartments by Plexiglas partitions containing an opening through which the mice could freely enter the 3 chambers. All testing was performed between the hours of 8 am and 1 pm. The test was conducted in two 10-minute phases. In phase I, the test mouse was allowed to explore the chambers for 10 minutes. Pilot experiments showed that mice did most of their chamber exploration and zone interaction in the first half of each block. Therefore, we report social preference as the time spent interacting socially (sniffing) in first 5 minutes of each phase. Each of the two outer chambers contained an empty, inverted stainless steel wire cup (Galaxy Cup, Spectrum Diversified Designs, Inc., Streetsboro, OH). Stranger mice were habituated to a wire cup for at least 30 minutes before use. In phase II, the test mouse was briefly removed, an unfamiliar mouse, age and sex matched, was placed under one of the wire cups and Lego blocks were placed under the other wire cup. The test mouse was then gently placed back in the arena and given an additional 10 minutes to explore. Room lighting for social behavior studies was 1-2 lux, measured using a Minolta IV F light meter. An overhead camera (Sony CCD Digital Ultra Pro Series, able to detect images down to 0.05 lux). and Ethovision v3 video tracking software (Noldus, Leesburg VA) were used to record the amount of time spent in each chamber and the number of entries into each chamber. In addition, a human observer, blinded to the treatment groups, scored time spent sniffing each wire cage, using Ethovision Observer software. Only male mice were tested. Stranger mice were used up to 4 times before new strangers were cycled in. The location (left or right) of the novel object and novel mouse alternated across subjects. To avoid chance differences in groups selected for single-dose treatment, the saline and poly(IC) exposure groups were each balanced according to their social

approach scores at 2.25 months of age prior to single-dose treatment with saline or suramin at 6.5 months. Results of social behavior testing are reported as the percent of time spent interacting with a stranger mouse (vs Lego blocks) in the first five minutes (mean +/- SEM). Social behaviors were evaluated at 2-4 days, and 5 weeks after suramin injection.

### **T-Maze**

The T-maze apparatus is constructed of black plexiglass. The protocol is adapted from Frye and Wolf<sup>2</sup>. The main stem is 45 cm long, 10 cm wide, and 24 cm high. Each side arm is 35 cm long, 10 cm wide, and 24 cm high. The side arms are separated from the stem by horizontal sliding doors. A start box, 8 cm in length, is also separated by a horizontal sliding door. Testing was conducted by an examiner that was blinded to the experimental groups, under low illumination, between 8 am and 1 pm. Only male animals were tested. Each mouse was tested in a session of 11 successive trials. The mice were not habituated to the maze. For the first trial only, one goal arm was closed off, forcing the mouse to choose the only open arm. Subsequent trials were by free choice. The chosen arm, and the time it takes for the mouse to choose (latency) were recorded. There was no confinement time in the chosen arm or in the start box. We confirmed that the saline and poly(IC) exposure groups had equivalent pre-treatment T-maze scores prior to single-dose treatment with saline or suramin. The percentage of alternated choices (mean +/- SEM) is reported. Spontaneous alternation was evaluated at 2-4 days, and 5 weeks after suramin injection.

### **Rotarod**

Training and testing were performed between the hours of 8 am and 1 pm using an accelerating rotarod protocol<sup>3</sup> (Economex Rotarod, Columbus Instruments fitted with a 4 cm diameter grooved plastic (not steel) spindle) as previously described<sup>4</sup> [ENREF\\_85](#). The plastic spindle is more slippery than grooved steel and results in shorter, but highly reproducible latencies. Only

male animals were tested. Prior to testing on an accelerating rod, mice were first trained at a fixed speed of 4 rpm. Each mouse was given up to 3 consecutive trials to achieve the endpoint of maintaining balance on the rotarod for at least 30 seconds. If a mouse was unsuccessful in the first 3 attempts, it was rested for 30 minutes, and then given another 3 attempts. Using this training protocol, all of the mice successfully maintained balance for 30 seconds within 2 training sessions. The acceleration phase testing was conducted over the subsequent 2 days, with 4 trials per day. Each mouse was individually placed on the rotarod at 4 rpm, which was then accelerated from 4 to 40 rpm over 5 minutes. The inter-trial time between repeat tests was 45 minutes. Latency to fall was recorded in seconds. Observers were blinded to treatment groups. Rotarod room lighting was 20-22 lux.

### **Light-Dark Box**

Anxiety-related and light-avoidance behaviors were tested in the light-dark box paradigm as previously described<sup>5</sup>. Briefly, the light-dark box consisted of two 18 × 20 × 18 cm chambers joined by a 6 × 6 cm door, with one side well-lit (850 lux) and the other side enclosed and darkened ( $\leq 5$  lux). At the start of the test, mice were placed in the light compartment and activity was recorded for 10 min. Percent time in the light chamber was analyzed by Ethovision Tracking Software (Noldus, Leesburg, VA, USA).

## **SOM TABLES AND LEGENDS**

**SOM Table S1. Biochemical Pathways Interrogated by Metabolomic Analysis.**

**SOM Table S2. Rank Ordered Metabolites by Univariate Analysis.**

**SOM Table S2 Legend.** Univariate analysis by 1-way ANOVA and a false discovery rate (FDR) threshold of 10% were used with pair-wise comparison and post hoc testing by Fisher's least significant difference method to identify metabolites that could discriminate between pairs of experimental groups. Metabolites shaded yellow were also identified by multivariate analysis (Figure 3d).

**SOM Table S3. Stable Isotope Internal Standards for LC-MS/MS.**

## SOM FIGURE LEGENDS

**SOM Figure S1. Single-Dose Correction of Behavioral Abnormalities.** (a) Social abnormalities in male MIA animals were found at the earliest ages of testing at 2.25 months of age. The four groups were balanced before treatment with saline or suramin. 2-way ANOVA followed by student's t-test with Bonferroni post-hoc correction was used to compare the time spent with mouse and cup in each experimental group. There was an interaction between prenatal exposure (Saline/Poly(IC)) and stimulus (mouse/cup); ( $F(3,39) = 9.28$ ;  $p < 0.0001$ ;  $N = 9-13$  per group). Student's t-test showed a social preference (mouse > cup) for the control (Saline) animals ( $p < 0.0001 = ****$ ) and no significant preference for the MIA (Poly(IC)) animals ( $p = ns$ ). (b) Single dose treatment of 6.5-month old MIA mice with suramin (PIC-Sur) restored normal social behavior ( $p < 0.0001 = ****$ ). Repeated measures ANOVA was used to test for the presence of interaction between prenatal exposure, drug treatment, and stimulus (mouse/cup). There was a prenatal exposure x drug treatment x stimulus interaction ( $F(1,39) = 9.34$ ;  $p < 0.01$ ) consistent with the observation that suramin benefited social behavior in the MIA animals, but had no effect on normal controls. Student's t-test with Bonferroni post-hoc correction was used to compare the time spent with mouse and cup in each experimental group. Saline treatment of MIA mice (PIC-Sal) had no effect; the time with mouse and cup were not significantly different ( $p = ns$ ). ( $N = 8-13$  per group). (c) After 5 weeks of suramin washout, the social behavior remained improved compared to saline-treated animals, but was decreased from the first week after treatment. Repeated measures ANOVA revealed an interaction between prenatal exposure and stimulus ( $F(1,39) = 6.35$ ;  $p < 0.05$ ) but no 3-way interaction between prenatal exposure, drug treatment, and zone. Student's t-test with Bonferroni post-hoc correction was used to compare the time spent with mouse and cup in each experimental group. This showed persistent absence of social preference in the saline-treated MIA animals (PIC-Sal;  $p = ns$ ), but still some residual social benefit of suramin 5-weeks after drug washout (PIC-Sur

mouse vs cup time;  $p < 0.01 = **$ ). (N = 8-13 per group). Values are expressed as means +/- SEM.

**SOM Figure S2. Light-Dark Box Behavior.** MIA and control animals were given a choice between spending time in the dark or exploring in the light. The percent time spent in the dark, measured over 10 minutes, was used as an index of avoidance or anxiety. No differences were found in the MIA model using our protocol ( $p = 0.96$  (ns); Student's t-test; Saline = 59.2 +/- 3.8%; Poly(IC) = 58.9 +/- 3.8%). Animals were 3.5-month old C57BL/6J MIA (poly(IC)-exposed) or control (saline-exposed) males. N = 19 Saline exposed and 25 Poly(IC).

### Online Supplementary References

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2. Frye CA, Walf AA. Effects of progesterone administration and APP<sup>swe</sup>+PSEN1<sup>Deltae9</sup> mutation for cognitive performance of mid-aged mice. *Neurobiology of learning and memory* 2008; **89**(1): 17-26.
3. Pallier PN, Drew CJ, Morton AJ. The detection and measurement of locomotor deficits in a transgenic mouse model of Huntington's disease are task- and protocol-dependent: influence of non-motor factors on locomotor function. *Brain research bulletin* 2009; **78**(6): 347-355.
4. Naviaux RK, Zolkipli Z, Wang L, Nakayama T, Naviaux JC, Le TP *et al.* Antipurinergic Therapy Corrects the Autism-Like Features in the Poly(IC) Mouse Model. *PloS one* 2013; **8**(3): e57380.
5. Toth M, Gresack JE, Bangasser DA, Plona Z, Valentino RJ, Flandreau EI *et al.* Forebrain-Specific CRF Over-Production During Development is Sufficient to Induce Enduring Anxiety and Startle Abnormalities in Adult Mice. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* 2013.

**SOM Table S1.** Biochemical Pathways Interrogated by Metabolomic Analysis.

| Pathway   | Metabolites | Pathway   | Metabolites |
|---|-------------|---|-------------|
| 1-Carbon, Folate, Formate, Glycine                          | 6           | Oxalate, Glyoxylate Metabolism                          | 3           |
| Amino acid metabolism not otherwise covered                 | 6           | Pentose Phosphate, Gluconate Metabolism                 | 11          |
| Amino-Sugar and Galactose Metabolism                        | 10          | Phosphate and Pyrophosphate Metabolism                  | 1           |
| Bile Salt Metabolism  | 4           | Phospholipid Metabolism                                 | 88          |
| Bioamines and Neurotransmitter Metabolism                   | 3           | Phytanic, Branch, Odd Chain Fatty Acids                 | 1           |
| Biopterin, Neopterin, Molybdopterin Metabolism              | 1           | Polyamine Metabolism                                    | 4           |
| Biotin (Vitamin B7) Metabolism                              | 1           | Purine Metabolism                                       | 48          |
| Branch Chain Amino Acid Metabolism                          | 7           | Pyrimidine Metabolism                                   | 35          |
| Cholesterol, Cortisol, Steroid Metabolism                   | 19          | SAM, SAH, Methionine, Cysteine, Glutathione Metabolism  | 22          |
| Endocannabinoid Metabolism                                  | 1           | Sphingolipid Metabolism                                 | 72          |
| Fatty Acid Oxidation and Synthesis                          | 7           | Taurine, Hypotaurine Metabolism                         | 2           |
| Food Sources, Additives, Preservatives, Colorings, and Dyes | 2           | Thyroxine Metabolism                                    | 1           |
| GABA, Glutamate, Arginine, Ornithine, Proline Metabolism    | 6           | Tryptophan, Kynurenine, Serotonin, Melatonin Metabolism | 6           |
| Glycolysis and Gluconeogenesis                              | 17          | Tyrosine and Phenylalanine Metabolism                   | 2           |
| Histidine, Histamine Metabolism                             | 2           | Urea Cycle  | 5           |
| Isoleucine, Valine, Threonine, or Methionine Metabolism     | 3           | Vitamin B1 (Thiamine) Metabolism                        | 4           |
| Ketone Body Metabolism                                      | 2           | Vitamin B12 (Cobalamin) Metabolism                      | 1           |
| Krebs Cycle   | 18          | Vitamin B2 (Riboflavin) Metabolism                      | 4           |
| Lysine Metabolism   | 2           | Vitamin B3 (Niacin/NAD) Metabolism                      | 7           |
| Microbiome Metabolism                                       | 32          | Vitamin B5 (Pantothenate) Metabolism                    | 1           |
| Nitric Oxide, Superoxide, Peroxide Metabolism               | 1           | Vitamin B6 (Pyridoxine) Metabolism                      | 6           |
| OTC and Prescription Pharmaceutical Metabolism              | 2           | Vitamin C (Ascorbate) Metabolism                        | 2           |
| Subtotal  | 152         | Subtotal  | 326         |
| <b>TOTAL Pathways and Chemical Sources</b>                  | <b>44</b>   | <b>TOTAL Metabolites</b>                                | <b>478</b>  |

## SOM Table S2. Rank Ordered Metabolites by Univariate Analysis.

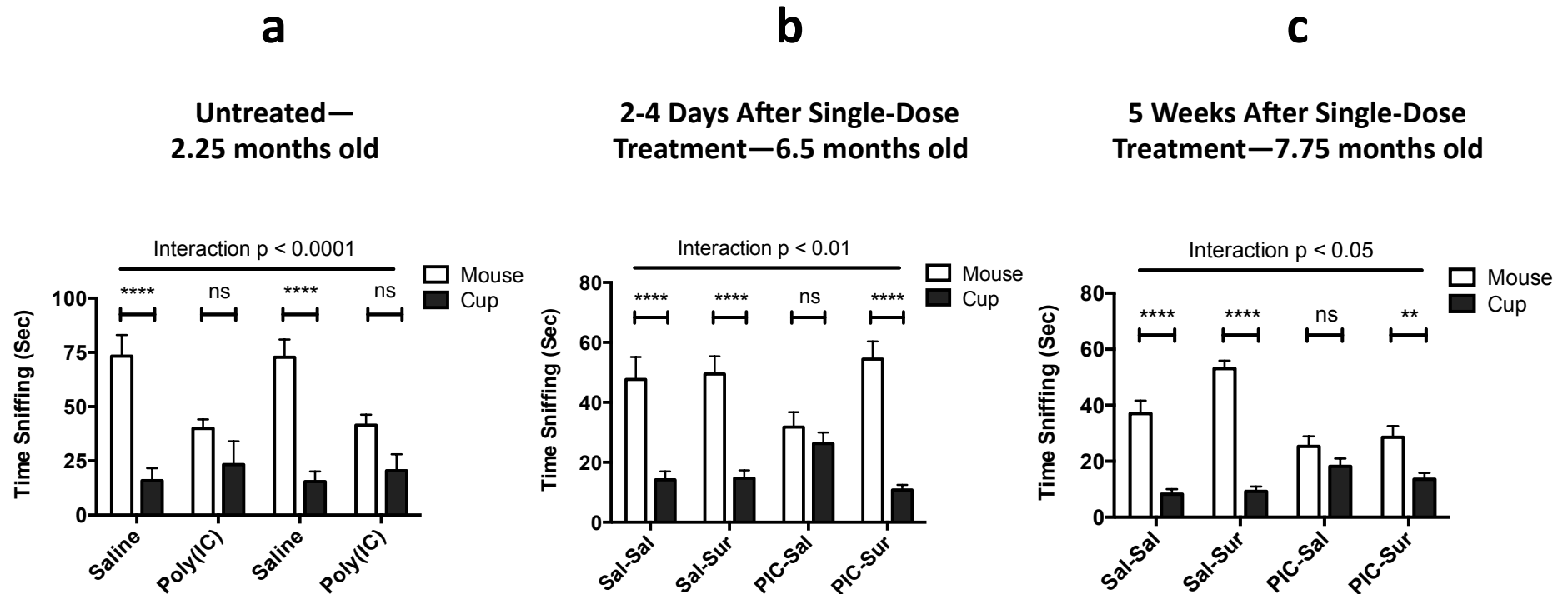
| No. | Pathway  | Metabolite                   | p-value    | -Log10(p) | FDR      | Fisher's LSD   |
|-----|--|------------------------------|------------|-----------|----------|--|
| 1   | Phospholipid Metabolism                                | Glycerophosphocholine        | 2.47E-07   | 6.6078    | 7.70E-05 | PIC Sur - PIC Sal; Sal Sal - PIC Sal; PIC Sur - PIC Sur W/O; Sal Sal - PIC Sur W/O |
| 2   | Cholesterol, Cortisol, Steroid Metabolism              | 24,25-Epoxycholesterol       | 3.22E-07   | 6.4917    | 7.70E-05 | PIC Sal - PIC Sur; PIC Sal - Sal Sal; PIC Sur W/O - PIC Sur; PIC Sur W/O - Sal Sal |
| 3   | Purine Metabolism                                      | dAMP                         | 5.11E-07   | 6.2918    | 7.88E-05 | PIC Sal - PIC Sur; PIC Sal - Sal Sal; PIC Sur W/O - PIC Sur; PIC Sur W/O - Sal Sal |
| 4   | Microbiome Metabolism                                  | Hydroxyphenylacetic acid     | 6.59E-07   | 6.1808    | 7.88E-05 | PIC Sal - PIC Sur; PIC Sal - Sal Sal; PIC Sur W/O - PIC Sur; PIC Sur W/O - Sal Sal |
| 5   | Krebs Cycle  | Oxaloacetic acid             | 0.00018264 | 3.7384    | 0.01746  | PIC Sur - PIC Sal; Sal Sal - PIC Sal; PIC Sur - PIC Sur W/O; Sal Sal - PIC Sur W/O |
| 6   | Phospholipid Metabolism                                | Palmitoylethanolamide        | 0.00024171 | 3.6167    | 0.018301 | PIC Sur - PIC Sal; Sal Sal - PIC Sal; PIC Sur - PIC Sur W/O; Sal Sal - PIC Sur W/O |
| 7   | Pyrimidine Metabolism                                  | Deoxyuridine                 | 0.00029313 | 3.5329    | 0.018301 | PIC Sal - PIC Sur; PIC Sal - Sal Sal; PIC Sur W/O - PIC Sur; PIC Sur W/O - Sal Sal |
| 8   | Tryptophan Metabolism                                  | Kynurenic acid               | 0.00032056 | 3.4941    | 0.018301 | PIC Sur - PIC Sal; Sal Sal - PIC Sal; PIC Sur - PIC Sur W/O; PIC Sur - Sal Sal     |
| 9   | Pyrimidine Metabolism                                  | Uridine                      | 0.00034459 | 3.4627    | 0.018301 | PIC Sur - PIC Sal; Sal Sal - PIC Sal; PIC Sur - PIC Sur W/O; Sal Sal - PIC Sur W/O |
| 10  | Purine Metabolism                                      | ATP                          | 0.00043906 | 3.3575    | 0.020987 | PIC Sur - PIC Sal; Sal Sal - PIC Sal; PIC Sur - PIC Sur W/O; Sal Sal - PIC Sur W/O |
| 11  | Purine Metabolism                                      | Adenine                      | 0.00060284 | 3.2198    | 0.025208 | PIC Sur - PIC Sal; Sal Sal - PIC Sal; PIC Sur - PIC Sur W/O; Sal Sal - PIC Sur W/O |
| 12  | Microbiome Metabolism                                  | 2,3-Dihydroxybenzoate        | 0.00063285 | 3.1987    | 0.025208 | PIC Sur - PIC Sal; Sal Sal - PIC Sal; PIC Sur - PIC Sur W/O; Sal Sal - PIC Sur W/O |
| 13  | Microbiome Metabolism                                  | 2-oxo-4-methylthiobutanoate  | 0.00071951 | 3.143     | 0.025357 | PIC Sur - PIC Sal; Sal Sal - PIC Sal; PIC Sur - PIC Sur W/O; Sal Sal - PIC Sur W/O |
| 14  | Pyrimidine Metabolism                                  | Thymine                      | 0.00074269 | 3.1292    | 0.025357 | PIC Sur - PIC Sal; Sal Sal - PIC Sal; PIC Sur - PIC Sur W/O; Sal Sal - PIC Sur W/O |
| 15  | Vitamin B6 (Pyridoxine) Metabolism                     | Nicotinate                   | 0.0010241  | 2.9897    | 0.032629 | Sal Sal - PIC Sal; PIC Sur - PIC Sur W/O; Sal Sal - PIC Sur W/O                    |
| 16  | Sphingolipid Metabolism                                | Ceramide 22:0                | 0.0010922  | 2.9617    | 0.032629 | PIC Sur - PIC Sal; Sal Sal - PIC Sal; PIC Sur - PIC Sur W/O; Sal Sal - PIC Sur W/O |
| 17  | Phospholipid Metabolism                                | PC(18:0/20:3)                | 0.0014321  | 2.844     | 0.037644 | PIC Sur - PIC Sal; Sal Sal - PIC Sal; PIC Sur - PIC Sur W/O; Sal Sal - PIC Sur W/O |
| 18  | Tryptophan Metabolism                                  | Quinolinic Acid              | 0.0014598  | 2.8357    | 0.037644 | Sal Sal - PIC Sal; Sal Sal - PIC Sur; Sal Sal - PIC Sur W/O                        |
| 19  | Glycolysis, Gluconeogenesis, Galactose Metabolism      | D-Fructose 6-phosphate       | 0.0017065  | 2.7679    | 0.037644 | PIC Sur - PIC Sal; Sal Sal - PIC Sal; PIC Sur - PIC Sur W/O; Sal Sal - PIC Sur W/O |
| 20  | Fatty Acid Oxidation and Synthesis                     | Oleic acid                   | 0.0017085  | 2.7674    | 0.037644 | PIC Sur - PIC Sal; PIC Sur - PIC Sur W/O; Sal Sal - PIC Sur W/O                    |
| 21  | Microbiome Metabolism                                  | Benzoic acid                 | 0.0017142  | 2.7659    | 0.037644 | Sal Sal - PIC Sal; Sal Sal - PIC Sur; Sal Sal - PIC Sur W/O                        |
| 22  | Pyrimidine Metabolism                                  | Carbamoyl-phosphate          | 0.0018628  | 2.7298    | 0.037644 | PIC Sal - PIC Sur W/O; PIC Sur - PIC Sur W/O; Sal Sal - PIC Sur W/O                |
| 23  | Vitamin B5 (Pantothenate) Metabolism                   | Pantothenic acid             | 0.0018832  | 2.7251    | 0.037644 | PIC Sal - PIC Sur; PIC Sal - Sal Sal; PIC Sur W/O - PIC Sur; PIC Sur W/O - Sal Sal |
| 24  | SAM, SAH, Methionine, Cysteine, Glutathione Metabolism | Dimethylglycine              | 0.0018901  | 2.7235    | 0.037644 | PIC Sur - PIC Sal; PIC Sur - PIC Sur W/O; PIC Sur - Sal Sal                        |
| 25  | Phospholipid Metabolism                                | N-oleoylethanolamine         | 0.0029363  | 2.5322    | 0.052436 | PIC Sur - PIC Sal; Sal Sal - PIC Sal; PIC Sur - PIC Sur W/O                        |
| 26  | Microbiome Metabolism                                  | Xanthosine                   | 0.0029631  | 2.5283    | 0.052436 | PIC Sur - PIC Sal; PIC Sur - PIC Sur W/O   |
| 27  | Phospholipid Metabolism                                | Ethanolamine                 | 0.003045   | 2.5164    | 0.052436 | PIC Sur - PIC Sal; PIC Sur - PIC Sur W/O; Sal Sal - PIC Sur W/O                    |
| 28  | Cholesterol, Cortisol, Steroid Metabolism              | 24-Dihydroxysterol           | 0.0030716  | 2.5126    | 0.052436 | PIC Sur - PIC Sal; Sal Sal - PIC Sal; PIC Sur - PIC Sur W/O; Sal Sal - PIC Sur W/O |
| 29  | Vitamin B6 (Pyridoxine) Metabolism                     | 4-Pyridoxic acid             | 0.0032599  | 2.4868    | 0.053732 | PIC Sur - PIC Sal; Sal Sal - PIC Sal; PIC Sur - PIC Sur W/O; Sal Sal - PIC Sur W/O |
| 30  | Purine Metabolism                                      | 7-methylguanosine            | 0.0035257  | 2.4528    | 0.056176 | PIC Sal - PIC Sur; PIC Sal - Sal Sal; PIC Sur W/O - PIC Sur; PIC Sur W/O - Sal Sal |
| 31  | Krebs Cycle  | Succinic acid                | 0.0039805  | 2.4001    | 0.059459 | PIC Sur - PIC Sal; Sal Sal - PIC Sal; PIC Sur - PIC Sur W/O; Sal Sal - PIC Sur W/O |
| 32  | Microbiome Metabolism                                  | 3-methylphenylacetic acid    | 0.0039805  | 2.4001    | 0.059459 | Sal Sal - PIC Sal; PIC Sur - PIC Sur W/O; Sal Sal - PIC Sur W/O                    |
| 33  | Tyrosine and Phenylalanine Metabolism                  | Tyrosine                     | 0.0043104  | 2.3655    | 0.062053 | PIC Sur - PIC Sal; Sal Sal - PIC Sal; PIC Sur - PIC Sur W/O; Sal Sal - PIC Sur W/O |
| 34  | Pentose Phosphate, Gluconate Metabolism                | D-Ribose-5-phosphate         | 0.0044273  | 2.3539    | 0.062053 | PIC Sur - PIC Sal; PIC Sur - PIC Sur W/O; Sal Sal - PIC Sur W/O                    |
| 35  | Krebs Cycle  | 2-Hydroxyglutarate           | 0.0045436  | 2.3426    | 0.062053 | PIC Sur - PIC Sal; Sal Sal - PIC Sal; PIC Sur - PIC Sur W/O                        |
| 36  | Microbiome Metabolism                                  | 3-Hydroxyanthranilic acid    | 0.0047418  | 2.3241    | 0.06296  | PIC Sal - PIC Sur; PIC Sal - Sal Sal; PIC Sur W/O - PIC Sur; PIC Sur W/O - Sal Sal |
| 37  | Branch Chain Amino Acid Metabolism                     | 4-methyl-2-oxopentanoic acid | 0.0050399  | 2.2976    | 0.065109 | PIC Sal - Sal Sal; PIC Sur - Sal Sal; PIC Sur W/O - Sal Sal                        |
| 38  | Bile Salt Metabolism                                   | Deoxycholic acid             | 0.0053945  | 2.268     | 0.067857 | Sal Sal - PIC Sal; Sal Sal - PIC Sur; Sal Sal - PIC Sur W/O                        |
| 39  | Fatty Acid Oxidation and Synthesis                     | Carnitine                    | 0.005777   | 2.2383    | 0.070579 | PIC Sal - PIC Sur; PIC Sur W/O - PIC Sur; Sal Sal - PIC Sur                        |
| 40  | Thyroxine Metabolism                                   | Diiodothyronine              | 0.0059062  | 2.2287    | 0.070579 | PIC Sur - PIC Sal; Sal Sal - PIC Sal; PIC Sur - PIC Sur W/O                        |
| 41  | Purine Metabolism                                      | Allantoin                    | 0.0065793  | 2.1818    | 0.076705 | PIC Sur - PIC Sal; Sal Sal - PIC Sal; PIC Sur - PIC Sur W/O; Sal Sal - PIC Sur W/O |
| 42  | Bile Salt Metabolism                                   | Taurodeoxycholic acid        | 0.00709    | 2.1494    | 0.08069  | Sal Sal - PIC Sal; Sal Sal - PIC Sur; Sal Sal - PIC Sur W/O                        |
| 43  | Microbiome Metabolism                                  | p-Hydroxybenzoate            | 0.0081414  | 2.0893    | 0.090502 | PIC Sal - PIC Sur; PIC Sal - Sal Sal   |
| 44  | Branch Chain Amino Acid Metabolism                     | Hydroxyisocaproic acid       | 0.0085126  | 2.0699    | 0.091299 | PIC Sur - PIC Sal; Sal Sal - PIC Sal; Sal Sal - PIC Sur W/O                        |
| 45  | SAM, SAH, Methionine, Cysteine, Glutathione Metabolism | Reduced glutathione          | 0.0085951  | 2.0658    | 0.091299 | Sal Sal - PIC Sal; Sal Sal - PIC Sur W/O   |
| 46  | Amino Acid Metabolism not otherwise covered            | Asparagine                   | 0.0088664  | 2.0523    | 0.092133 | Sal Sal - PIC Sal; PIC Sur - PIC Sur W/O; Sal Sal - PIC Sur W/O                    |



**SOM Table S3. Stable Isotope-Labeled Internal Standards for LC-MS/MS.**

| Polarity | Isotope Standards                                   | Stock                       | Q1     | Q3     | DP      | EP     | CE     | CXP    |
|----------|---|-----------------------------|--------|--------|---------|--------|--------|--------|
|          |   | Concentration<br>( $\mu$ M) |        |        |         |        |        |        |
| Positive | L-Alanine (2,3,3,3-D4)                              | 10.102                      | 94.05  | 48.1   | 54.45   | 10     | 11.08  | 11.16  |
| Positive | L-Phenylalanine (ring-13C6)                         | 10.000                      | 172.08 | 126    | 78.1    | 10     | 21     | 26.93  |
| Positive | L-Leucine (5,5,5,-D3)                               | 10.450                      | 135.1  | 89.2   | 70.93   | 10     | 10.87  | 20.1   |
| Positive | L-Valine (D8)                                       | 10.184                      | 126.05 | 77.2   | 70.93   | 10     | 18.64  | 20.1   |
| Negative | L-Arginine:HCl (5-13C,4,4,5,5-D4)                   | 11.145                      | 178.11 | 145.18 | -49.74  | -11.19 | -13.93 | -20.65 |
| Negative | L-Citrulline (5,5,-D2)                              | 10.164                      | 176.1  | 133.1  | -88.095 | -9.95  | -21.1  | -21.95 |
| Positive | DL-Glutamic acid (2,4,4-D3)                         | 10.409                      | 151.06 | 115    | 115.4   | 9.04   | 9.97   | 12.93  |
| Positive | L-Tyrosine (ring-13C6)                              | 10.204                      | 188.08 | 142    | 43.25   | 10.68  | 16.06  | 18.2   |
| Positive | L-Ornithine:HCl (5,5-D2)                            | 9.980                       | 135.09 | 72     | 43.82   | 10.8   | 33.58  | 17.29  |
| Positive | L-Methionine (methyl-D3)                            | 10.409                      | 153.05 | 63.9   | 59.88   | 10     | 29.18  | 26.11  |
| Negative | L-Aspartic Acid (2,3,3-D3)                          | 10.061                      | 135.04 | 91.1   | -50.16  | -8.32  | -21.11 | -38.99 |
| Positive | Glycine (2-13C, 15N)                                | 51.125                      | 79.03  | 61.6   | 78      | 12     | 5      | 11.4   |
| Positive | L-Glutamine-amide-15N                               | 10.000                      | 148.07 | 84.1   | 35.59   | 7.29   | 26.53  | 15.19  |
| Negative | D-Glucose-13C6                                      | 10.000                      | 185.07 | 61     | -36.2   | -10    | -23.1  | -5.8   |
| Negative | D-Fructose-13C6                                     | 10.000                      | 185.07 | 61.01  | -36.2   | -10    | -23.1  | -5.8   |
| Negative | Alpha-Ketoisocaproic acid (1-13C, 99%)              | 10.000                      | 130.06 | 85     | -47.37  | -10.18 | -10.21 | -19.32 |
| Negative | Uric acid-1,3-15N2                                  | 10.000                      | 169.03 | 125    | -95     | -10    | -20    | -4     |
| Positive | Creatinine-(methyl-13C)                             | 10.000                      | 115.06 | 87.1   | 31      | 10     | 15     | 4      |
| Negative | Sucrose-13C12                                       | 10.000                      | 353.12 | 92.02  | -127.19 | -12.3  | -29.8  | -15.05 |
| Positive | Glycerol-13C3                                       | 10.000                      | 96.05  | 59.2   | 99.65   | 10.93  | 20.86  | 11.89  |
| Positive | L-carnitine (N-trimethyl-D9)                        | 20.905                      | 171.11 | 103.03 | 72.37   | 10     | 27.29  | 15     |
| Positive | L-Acetylcarnitine(N-methyl-D3)                      | 5.216                       | 207.12 | 85.02  | 61.16   | 8.33   | 42.38  | 12.56  |
| Positive | L-Propionylcarnitine (N-methyl-D3)                  | 1.041                       | 221.13 | 159    | 65.61   | 13.02  | 18.6   | 29.67  |
| Positive | L-Butyrylcarnitine (N-methyl-D3)                    | 1.041                       | 235.15 | 173.1  | 51.3    | 9.69   | 17.1   | 8.8    |
| Positive | L-Isovalerylcarnitine (N-trimethyl-D9)              | 1.081                       | 255.17 | 187.1  | 81.73   | 10.65  | 20.49  | 24.89  |
| Positive | L-Octanoylcarnitine (N-methyl-D3)                   | 1.000                       | 291.22 | 85.1   | 85.23   | 10.1   | 29.52  | 15.12  |
| Positive | L-Myristoylcarnitine (N-trimethyl-D9)               | 1.027                       | 381.31 | 84.9   | 87.75   | 10.08  | 29.38  | 14.5   |
| Positive | L-Palmitoylcarnitine (N-methyl-D3)                  | 2.054                       | 403.34 | 85     | 49.64   | 10.22  | 35.83  | 16.99  |
| Positive | L-carnitine (mono):ClO4, O-Glutaryl (N-methyl-D3)   | 1.889                       | 279.14 | 85.1   | 55.82   | 11.71  | 29.35  | 14.83  |
| Positive | L-carnitine:ClO4, 3-Hydroxyisovaleryl (N-methyl-D3) | 1.028                       | 265.16 | 85.2   | 76.11   | 10.98  | 34.09  | 20     |
| Positive | L-carnitine:HCl, O-Dodecanoyl(N,N,N-Trimethyl-D9)   | 1.000                       | 353.28 | 84.8   | 92.11   | 9.53   | 56.38  | 17.6   |
| Positive | L-carnitine:HCl, O-Octadecanoyl(N-methyl-D3)        | 2.042                       | 431.37 | 369.4  | 69.18   | 10.36  | 29.04  | 17.27  |
| Positive | Cholesterol-d7                                      | 50.000                      | 376.36 | 161.1  | 110     | 10     | 30.7   | 14     |
| Positive | PC (16:0/16:0)-d62                                  | 20.000                      | 796.58 | 184    | 32.85   | 10.11  | 43.27  | 27.02  |
| Negative | Trypan blue   | 20.000                      | 435.03 | 185    | -144.58 | -8.67  | -57.8  | -20.94 |

# SOM Figure S1. Single-Dose Correction of Behavioral Abnormalities.



# SOM Figure S2

