Supporting Information

Naviaux RK, Curtis B, Li K, Naviaux JC, Bright AT, Reiner G, Westerfeld M, Goh S, Alaynick WA, Wang L, Capparelli EV, Adams C, Sun J, Jain S, He F, Arellano DA, Mash L, Chukoskie L, Lincoln A, Townsend J. **Low-dose suramin in autism spectrum disorder: a small, phase I/II, randomized clinical trial**. 2017, *Annals of Clinical and Translational Neurology*.

Contents

1. **Supplemental Materials and Methods**
2. **Supplemental Results**
3. **Supplemental References**
4. **Supplemental Figures Legends**
5. **Supplemental Tables**
6. **Supplemental Figures**
7. **Supplemental Data**

    S1. Clinical Global Impression (CGI) questionnaire

    S2. Social stories to accompany the storyboard

Supplemental Materials and Methods

Diagnostic and Outcome Procedures

Examiner-based outcomes (ADOS and EOWPVT) were assessed at 2-days and 6-weeks after the infusion. Parent-based outcomes (ABC, ATEC, CGI, and RBQ) were assessed at 7-days and 6-weeks after the infusion. To minimize the effects of natural behavioral variability, the parents were instructed to mark a behavior as changed only if it was persistently changed for at least 1 week. Storyboards and accompanying social stories were created to illustrate each step of the study for parents to review with each child before the study (Figure S1, and Supplemental Data S2).

Safety and Adverse Event Monitoring

Blood and urine for safety and toxicity monitoring were collected immediately before the infusion, 1 hour after the infusion, 2 days after, and 45 days after the infusion. Vital signs and anthropomorphic measurements were also collected. Safety surveillance included 18 vital sign and anthropometric features, 19 complete blood count (CBC) parameters, 20 blood chemistry measures, 3 thyroid and cortisol measures, and 5 lipid measures at the 5 time points. 24 urinalysis features were measured at 4 times: baseline, pre-infusion, 2-days post-infusion, and 45-days post-infusion.

Verification of Data Completeness and Transcription Accuracy

Standardized questionnaire responses and the ADOS-2 and EOWPVT scores (5,490 cells of data) were compiled in spreadsheets from the original hard copy forms and from the electronic medical records. A total of 87 cells (1.6%) of the 5,490 outcome scores were either left blank,

asked about a symptom that did not apply, or were missing. One participant missed the 6-week ADOS and EOWPVT evaluations because of scheduling difficulties. His 2-day results were used as an estimate of his 6-week scores. ADOS scores remained significant when this subject was dropped from the analysis (Figure S4T). EOWPVT results were also unchanged (Figure S4X). The 4,210 cells of laboratory and vital sign data were also collected and reviewed. When specific cells of data were found to be missing, they were manually confirmed by inspection of the original questionnaire, laboratory results, and clinical data sheets. A random generator program was written that randomly selected 5% of the data. These randomly selected cells of data that were then manually checked for transcription accuracy by reviewing the hard copy responses and Red Cap electronic medical records.

Standardized Testing and Questionnaires

Two observational examinations were performed by a clinician at 3 time points: baseline (56 ± 8 days; mean ± SEM; before the infusion), 2-days post-infusion, and 6-weeks post-infusion. The two examiner-based metrics were the Autism Diagnostic Observation Schedule, 2nd edition (ADOS-2)[1, 2], with video and audio files recorded on 3 cameras, and the Expressive One Word Picture Vocabulary Testing (EOWPVT)[3]. Both of these observational metrics were administered by a trained and certified examiner using approved test materials. Three standardized questionnaires were completed by parents at 3 time points: baseline, 7-days post-infusion, and 6-weeks post-infusion. The three standardized questionnaires completed by parents were the 58-question Aberrant Behavior Checklist (ABC)[4], the 75-item Autism Treatment Evaluation Checklist (ATEC)[5, 6], and the 33-item repetitive behavior questionnaire (RBQ)[7]. Parents were asked to complete these three instruments with reference to how their child behaved in the

previous 7 days. At the end of the six weeks, we included a 24-question Clinical Global Impression (CGI)[8] questionnaire (Supplementary Data S1). In addition, parents were asked to list the 3 top behaviors or symptoms that they observed to be most changed over the previous 6-weeks. To minimize the misinterpretation of natural day-to-day variations in symptoms, parents were asked to mark a symptom as changed in the 6-week CGI only if it had lasted for at least 1 week.

Storyboards and Social Stories

We commissioned a graphic artist to prepare a storyboard of each step of the procedure (Figure S1). The panel contents and color schemes were reviewed, and revisions recommended, by a 16-year old artist with Asperger syndrome to optimize the informational value and minimize any sensory issues. Next, our developmental neuropsychologist created social stories to accompany each panel of the storyboard. The social stories are shown in Supplementary Data S2.

Phone Interviews, Parent Reports, and Clinical Observations

Scripted phone interviews were conducted daily for the first week, then weekly until the completion of the study for each child 6-weeks after the infusion. Parents also kept study journals throughout the six weeks to document their observations. These scripted and narrative observations were used to permit discovery of any changes in ASD, behavior, or constitutional symptoms such as sleep and appetite, or any adverse or unanticipated events. The parent reports also provided insight regarding the timing and pattern of the responses after the infusion that were not predicted prior to the study, and were not adequately captured by the scheduled observations.

Daily Calls. Parents were contacted by phone on days 1-7 after the infusion to ensure close follow-up and to provide the opportunity for parents to report any positive or negative observations. These calls followed the script below:

"Hi. This is _____ (state your name) at UCSD. This is our daily follow-up call to see how you and your son are doing as part of the autism study."

1. How have things been going since the infusion? Any changes since yesterday?
2. Have there been any improvements? What things are most improved?
3. Have there been any setbacks, or negative things you've noticed? What are these?
4. How is he eating?
5. How is he sleeping?
6. Are there any problems, suggestions, or concerns that I can pass on to the doctors or a nurse?

Weekly Calls. Parents were called weekly on days 14, 21, 28, and 35 after the infusion to ensure close follow-up and to provide the opportunity for parents to report any positive or negative observations. These calls followed the script below:

"Hi. This is _____ (state your name) at UCSD. This is our weekly follow-up call to see how you and your son are doing as part of the autism study."

1. How have things been going since the infusion? Any changes since last week?
2. Have there been any improvements? What things are most improved?
3. Have there been any setbacks, or negative things you've noticed? What are these?
4. How is he eating?
5. How is he sleeping?
6. Are there any problems, suggestions, or concerns that I can pass on to the doctors or a nurse?

Clinical Global Impression (CGI)

We developed a 24-question Clinical Global Impression (CGI) instrument designed to assess the core symptoms of autism spectrum disorders and some of the most common comorbid features (Supplementary Material A1). The CGI instrument scoring system was the traditional 7-point,

CGI-Improvement scale[8]. In this scale, the historian gives a score of 0 if the symptom "was never a problem", a 1 for "very much improved", a 4 for "no change", and a 7 for "very much worse". In addition to the 24 structured questions, we asked the parents to write in the top 3 symptoms or behaviors that were most changed over the 6 weeks since the suramin infusion (Supplementary Material A1). This hybrid design of structured and open-ended responses permitted us to capture a large number of clinical outcomes associated with single-dose suramin treatment.

Metabolomics

Targeted, broad-spectrum, plasma metabolomic analysis of 610 metabolites from 63 biochemical pathways was performed by high performance liquid chromatography and tandem mass spectrometry (LC-MS/MS) as described[9] with minor modifications. 431 metabolites were above the lower limit of quantitation (LLOQ) in this study. Venous blood was collected between the hours of 8 am and 5 pm, at least 3 hours after the last meal, into lithium-heparin vacutainer tubes (BD #367884). Plasma was separated by centrifugation at 900g x 10 minutes at room temperature within one hour of collection. The resulting fresh lithium-heparin plasma was transferred to labeled 1.2 ml or 2.0 ml externally threaded, cryotubes with a minimum headspace air gap for storage at -80˚C for analysis. Samples were analyzed on an AB SCIEX QTRAP 5500 triple quadrupole mass spectrometer equipped with a Turbo V electrospray ionization (ESI) source, Shimadzu LC-20A UHPLC system, and a PAL CTC autosampler. Typically, 90 µl of plasma was thawed on ice and transferred to a 1.7 ml Eppendorf tube. Five (5.0) µl of a cocktail containing 25-35 commercial stable isotope internal standards, and 5.0 µl of 57 stable isotope internal standards that were custom-synthesized in *E. coli NCM3722, Caenorhabditis elegans N2,*

and *Komagataella phaffii* (ATCC 76273; formerly known as *Pichia pastoris*) by metabolic labeling with ^{13}C-glucose and ^{13}C-bicarbonate, were added, mixed, and incubated for 10 min at 20˚C to permit small molecules and vitamins in the internal standards to associate with plasma binding proteins. Macromolecules (protein, DNA, RNA, glycans, etc.) were precipitated by extraction with 4 volumes (400 µl) of cold (-20˚C), acetonitrile:methanol (50:50) (LCMS grade, Cat# LC015-2.5 and GC230-4, Burdick & Jackson, Honeywell), vortexed vigorously, and incubated on crushed ice for 10 min, then removed by centrifugation at 16,000g x 10 min at 4˚C. The supernatants containing the extracted metabolites and internal standards in the resulting 40:40:20 solvent mix of acetonitrile:methanol:water were transferred to labeled cryotubes and stored at -80˚C for LC-MS/MS analysis.

LC-MS/MS analysis was performed by scheduled multiple reaction monitoring (sMRM) under Analyst v1.6.2 software control in both negative and positive mode with rapid polarity switching (50 ms). Nitrogen was used for curtain gas (set to 30), collision gas (set to high), ion source gas 1 and 2 (set to 35). The source temperature was 500˚C. Spray voltage was set to -4500 V in negative mode and 5500 V in positive mode. The values for Q1 and Q3 mass-to-charge ratios (m/*z*), declustering potential (DP), entrance potential (EP), collision energy (CE), and collision cell exit potential (CXP) were determined and optimized for each MRM for each metabolite. Ten microliters of extract was injected by PAL CTC autosampler via a 10 µl stainless steel loop into a 250 mm × 2.0 mm, 4µm polymer based NH2 HPLC column (Asahipak NH2P-40 2E, Showa Denko America, Inc., NY) held at 25°C for chromatographic separation. The mobile phase was solvent A: 95% water with 20 mM $(NH_4)_2CO_3$ (Sigma, Fluka Cat# 74415-250G-F), 5% acetonitrile, and 38 mM NH_4OH (Sigma, Fluka Cat# 17837-100ML), final pH 9.75; solvent B:

100% acetonitrile. Separation was achieved using the following gradient: 0-3.5 min: 95%B, 3.6-8 min: 85% B, 8.1-13 min: 75% B, 13.5–35 min: 0% B, 36–46 min: 95% B, 46.1 min: end. The flow rate was 200 µl/min. Pump pressures ranged from 920-2600 psi over the course of the gradient. All the samples were kept at 4°C during analysis. The chromatographic peaks were identified using MultiQuant (v3.0, Sciex), confirmed by manual inspection, and the peak areas integrated.

Suramin Quantitation

Suramin concentrations were measured by LC-MS/MS as previously described with modifications[10]. Plasma suramin samples were collected at 1 hour, 2 days and 42 days post-infusion. Heparinized plasma, 90 µl was used. Ten (10) µl of 50 µM stock of trypan blue was added to achieve an internal standard concentration of 5 µM. This was incubated at room temperature for 10 min to permit metabolite interaction with binding proteins, then extracted with 4 volumes (400 µl) of pre-chilled methanol-acetonitrile (50:50) to produce a final concentration of 40:40:20 (methanol:acetonitrile:H_2O), and precipitated on ice for 10 minutes. The samples were deproteinated and macromolecules removed by precipitation on crushed ice for 10 min. The mixture was centrifuged at 16,000g for 10 min at 4°C and the supernatant was transferred to a new tube and kept at -80°C for further LC-MS/MS analysis.

Suramin was analyzed on an AB SCIEX QTRAP 5500 triple quadrupole mass spectrometer equipped with a Turbo V electrospray ionization (ESI) source, Shimadzu LC-20A UHPLC system, and a PAL CTC autosampler. Ten microliters of extract were injected onto a Kinetix F5 column (100 × 2.1 mm, 2.6 µm; Phenomenex, CA) held at 30°C for chromatographic separation.

The mobile phase A was water with 20 mM ammonium acetate (NH_4OAC) (pH 7) and mobile phase B was methanol with 20 mM NH_4OAC (pH 7). Elution was performed using the following gradient: 0-1.5 min-0% B, 1.6-3 min-15% B, 3.1-7 min-60% B, 7.1-13 min-100% B, 14 min-0% B, 18 min-0% B, 18.1 minute-end. The flow rate was 400 µl/min. All the samples were kept at 4°C during analysis. Suramin and trypan blue were detected using MRM scanning mode with the dwell time of 180 ms. MRM transitions for the doubly-charged form of suramin were 647.0 m/z for the (Q1) precursor and 382.0 m/z for the (Q3) product. MRM transitions for trypan blue were 435.2 (Q1) and 185.0 (Q3). Absolute concentrations of suramin were determined using a standard curve prepared in plasma to account for matrix effects, and the peak area ratio of suramin to the internal standard trypan blue. The declustering potential (DP), collision energy (CE), entrance potential (EP) and collision exit potential (CXP) were -104, -9.5, -32 and -16.9, and -144.58, -7, -57.8 and -20.94, for suramin and trypan blue, respectively. The ESI source parameters were set as follows: source temperature 500 °C; curtain gas 30; ion source gas 1, 35; ion source gas 2 35; spray voltage -4500 V. Analyst v1.6 was used for data acquisition and analysis.

Supplemental Results

Safety Monitoring and Adverse Events

The rash caused by suramin in this study was not raised and did not itch. It was not urticarial. The children did not appear to notice it. Any residual rash was covered by clothing and not visible on exposed skin at the 2-day evaluation. Parents were instructed not to discuss it with the neuropsychology team to decrease the chance of examiner bias. Video camera records of the

ADOS testing confirmed the absence of any visible rash. The rash was a known risk of suramin treatment that was described in the informed consent documents.

Pharmacokinetics

Additional pharmacokinetic results are illustrated in Table S1. Although no behavioral outcomes were significant at 2 days after infusion, we found that 28 biochemical pathways were changed by suramin 2-days after the infusion (Table S2). Twenty-two of these (79%) remained changed at the 6-week time point (see Table 3). The rank order of metabolites most changed at day 2, and their associated metabolic pathway is illustrated in Figure S2. The full list of 61 metabolites on day 2 and 48 metabolites at 6-weeks that were significantly changed by suramin appears in Tables S3-S4. A wallchart-style biochemical pathway map was created in Cytoscape to illustrate the organization of metabolites that were increased and decreased by suramin treatment (Figure S3).

Pharmacometabolomics

The small number of subjects in this trial precluded conventional treatment group analysis because of high false discovery rates associated with measuring 431 metabolites in groups with just 5 subjects. However, by using each child as their own control in a paired analysis of pre-infusion and post-infusion results, the pharmacometabolomic effects of suramin could be characterized (see Table 3 and Figures 4-5, Table S2 and Figure S2).

Treatment Outcomes

ADOS comparison scores were improved in the suramin treatment group at 6-weeks (Figure S4AB) but were unchanged in the saline group (Supplemental Figure S4AF). ADOS scores at 2-days after treatment were not changed (Figure S4E). EOWPVT scores were not changed (Figure S4I). Secondary outcomes included Aberrant Behavior Checklist (ABC), Autism Treatment Evaluation Checklist (ATEC), the Clinical Global Impression (CGI), and the Repetitive Behavior Questionnaire (RBQ). Suramin treatment was associated with improvements in the ABC, ATEC, and CGI, but not in the RBQ (Figure S4). Three of 24 symptoms covered in the CGI were significant (Figure S4aa). Parents were also asked to specify the three top, most-changed behaviors as an unstructured component of the CGI at 6-weeks after the infusion. Five symptoms were named that achieved statistically significant results. The most-changed behaviors were social communication and play, speech and language, calm and focus, stims or stereotypies, and coping skills (Figure S4bb).

Supplemental References

1. Lord C, Risi S, Lambrecht L, et al. The Autism Diagnostic Observation Schedule—Generic: A standard measure of social and communication deficits associated with the spectrum of autism. Journal of autism and developmental disorders. 2000;30(3):205-23.
2. Lord C, Rutter M, DiLavore P, Risi S, Gotham K, Bishop S. Autism Diagnostic Observation Schedule–2nd edition (ADOS-2). Los Angeles, CA: Western Psychological Corporation. 2012.
3. Adams-Chapman I, Bann C, Carter SL, Stoll BJ, Network NNR. Language outcomes among ELBW infants in early childhood. Early Hum Dev. 2015 Jun;91(6):373-9.
4. Kaat AJ, Lecavalier L, Aman MG. Validity of the aberrant behavior checklist in children with autism spectrum disorder. Journal of autism and developmental disorders. 2014;44(5):1103-16.
5. Geier DA, Kern JK, Geier MR. A Comparison of the Autism Treatment Evaluation Checklist (ATEC) and the Childhood Autism Rating Scale (CARS) for the Quantitative Evaluation of Autism. J Mental Health Research in Intellectual Disabilities. 2013;6:255-67.
6. Rimland B, Edelson S. Autism treatment evaluation checklist: statistical analyses. Autism Research Institute. 2000.
7. Honey E, McConachie H, Turner M, Rodgers J. Validation of the repetitive behaviour questionnaire for use with children with autism spectrum disorder. Research in Autism Spectrum Disorders. 2012;6(1):355-64.
8. Busner J, Targum SD. The clinical global impressions scale: applying a research tool in clinical practice. Psychiatry (Edgmont). 2007 Jul;4(7):28-37.
9. Naviaux RK, Naviaux JC, Li K, et al. Metabolic features of chronic fatigue syndrome. Proceedings of the National Academy of Sciences of the United States of America. 2016 Sep 13;113(37):E5472-80.
10. Naviaux JC, Schuchbauer MA, Li K, et al. Reversal of autism-like behaviors and metabolism in adult mice with single-dose antipurinergic therapy. Translational psychiatry. 2014;4:e400.
11. Pickles A, Le Couteur A, Leadbitter K, et al. Parent-mediated social communication therapy for young children with autism (PACT): long-term follow-up of a randomised controlled trial. Lancet. 2016 Nov 19;388(10059):2501-9.

Supplemental Figure Legends

1. **Figure S1.** Storyboard illustration of each step of the infusion day visit.
2. **Figure S2.** Suramin pharmacometabolomics. Rank order of metabolites and pathways that were changed by suramin at 2-days after treatment.
3. **Figure S3.** Suramin pharmacometabolomics pathway visualization. (A) After 2 days. (B) After 6 weeks. Metabolites indicated in red are increased, and those in green are decreased compared to controls (see z-score scale in upper right).
4. **Figure S4.** Outcomes. (A) 6-week ADOS Comparison Scores by 2-Way ANOVA. (B) 6-Week ADOS Comparison Score Improvement after Suramin. (C) 6-Week ADOS Social Affect Score Improvement after Suramin. (D) 6-Week ADOS Restricted and Repetitive Behavior Score Improvement after Suramin. (E) 2-Day ADOS Comparison Scores were not changed. (F) No change in 6-Week ADOS Scores in subjects receiving saline placebo. (G) No change in 6-Week ADOS Social Affect Scores in subjects receiving placebo. (H) No change in 6-Week ADOS Restricted and Repetitive Behavior Scores in subjects receiving placebo. (I) No change in 6-week Expressive One Word Picture Vocabulary scores. (J) 7-Day improvement in ABC stereotypy scores after suramin. (K) 6-week Improvement in ABC stereotypy scores after suramin. (L) 7-Day Improvement in ATEC total scores after suramin. (M) No change in 6-week EOWPVT scores after saline. (N) No change in 7-day ABC stereotypy scores after saline. (O) No change in 6-week ABC stereotypy scores after saline. (P) No change in 7-day ATEC total scores after saline. (Q) Improved ATEC speech, language, and communication scores 7-days after suramin. (R) Improved ATEC sociability scores 7-days after suramin. (S) Improved ATEC speech, language, and communication scores 6-weeks after suramin. (T) Improved ADOS comparison scores after dropping a

subject who missed the 6-week visit (N = 4). (U) No change in 7-day ATEC speech, language, and communication after saline. (V) No change in 7-day ATEC sociability after saline. (W) No change in 6-week ATEC speech, language, and communication scores 6-weeks after saline (X) No change in EOWPVT scores after dropping subject who missed the 6-week visit (N = 4). (Y) No change in 2-day ADOS scores after suramin. (Z) No change in 6-week RBQ total scores after suramin. (aa) Improved core symptoms of ASD and other behaviors by CGI at 6-weeks after suramin. P values: * = 0.05; ** = 0.01; *** = 0.001. (bb) Top 3, most-changed symptoms named by parents in the 6-week CGI. (cc) No change in 2-day ADOS scores after saline. (dd) No change in 6-week RBQ total scores after saline.

Supplemental Tables

1. **Table S1.** Single-dose suramin pharmacokinetics.
2. **Table S2.** Suramin pharmacometabolomics. Pathways changed at 2-days.
3. **Table S3.** Suramin pharmacometabolomics. Metabolites changed at 2-days.
4. **Table S4.** Suramin pharmacometabolomics. Metabolites changed at 6-weeks.

Supplemental Data

1. **S1.** Clinical Global Impression (CGI) questionnaire.

2. **S2.** Social Stories to Accompany the Storyboard Panels Describing Each Step of the Infusion Day Visit.

Table S1. Single-dose suramin pharmacokinetics.

Pair Block	ID	Age (yrs)	Height (m)	Weight (kg)	BSA* (m^2)	20 mg/kg Dose (mg)	Dose (mg/m^2)	1-Hour Plasma Conc (µM)	2-Day Plasma Conc (µM)	45-Day Plasma Conc (µM)	Plasma Half-Life (days)
1	001	11	1.395	34.4	1.15	680	591	101.2	13.2	0.96	12.6
2	007	5	1.189	22.9	0.87	460	529	87.9	11.9	1.67	14.7
3	014	14	1.74	54.7	1.63	1000	613	110.9	10.6	1.04	14.9
4	012	6	1.18	23.1	0.87	460	529	118.6	13.8	2.28	16.5
5	005	7	1.271	25.1	0.95	500	526	101.8	10.6	1.76	15.0
						Mean:	558	104.1	12.0	1.54	14.7
						sd:	41	11.6	1.5	0.5	1.4

*Mosteller method. BSA: body surface area.

Table S2. Suramin pharmacometabolomics. Pathways changed at 2-days.

No.	Pathway Name	Measured Metabolites in the Pathway (N)	Expected Pathway Proportion (P = N/431)	Expected Hits in Sample of 61 (P * 61)	Observed Hits in the Top 61 Metabolites	Fold Enrichment (Obs/Exp)	Impact (Sum VIP Score)	Fraction of Impact (VIP Score) Explained (% of 119.7)	Increased	Decreased
1	Purine Metabolism	26	0.060	3.7	9	2.4	17.6	15%	4	5
2	Bile Salt Metabolism	6	0.014	0.8	4	4.7	11.9	10%	4	0
3	Microbiome Metabolism	18	0.042	2.5	4	1.6	9.3	8%	4	0
4	Branch Chain Amino Acid Metabolism	12	0.028	1.7	4	2.4	7.3	6%	4	0
5	Eicosanoid and Resolvin Metabolism	13	0.030	1.8	4	2.2	7.1	6%	0	4
6	Phospholipid Metabolism	74	0.172	10.5	3	0.3	5.7	5%	0	3
7	SAM, SAH, Methionine, Cysteine, Glutathione	15	0.035	2.1	3	1.4	5.6	5%	2	1
8	GABA, Glutamate, Arginine, Ornithine	6	0.014	0.8	3	3.5	4.7	4%	3	0
9	Pyrimidine Metabolism	9	0.021	1.3	2	1.6	4.3	4%	1	1
10	Glycolysis and Gluconeogenesis Metabolism	7	0.016	1.0	2	2.0	4.3	4%	2	0
11	Gamma-Glutamyl and other Dipeptides	2	0.005	0.3	2	7.1	3.8	3%	2	0
12	Sphingomyelin Metabolism	36	0.084	5.1	2	0.4	3.6	3%	0	2
13	Bioamines and Neurotransmitter Metabolism	9	0.021	1.3	2	1.6	3.3	3%	0	2
14	Krebs Cycle	9	0.021	1.3	2	1.6	3.3	3%	2	0
15	Vitamin D (Calciferol) Metabolism	3	0.007	0.4	1	2.4	3.1	3%	0	1
16	Cardiolipin Metabolism	7	0.016	1.0	2	2.0	3.1	3%	2	0
17	Glycosphingolipid Metabolism	12	0.028	1.7	1	0.6	2.1	2%	1	0
18	Taurine, Hypotaurine Metabolism	2	0.005	0.3	1	3.5	2.0	2%	0	1
19	Nitric Oxide, Superoxide, Peroxide	2	0.005	0.3	1	3.5	1.9	2%	0	1
20	Histidine, Histamine, Carnosine Metabolism	4	0.009	0.6	1	1.8	1.8	2%	1	0
21	Tyrosine and Phenylalanine Metabolism	3	0.007	0.4	1	2.4	1.8	2%	1	0
22	Fatty Acid Oxidation and Synthesis	37	0.086	5.2	1	0.2	1.8	2%	0	1
23	Cholesterol, Cortisol, Non-Gonadal Steroid	16	0.037	2.3	1	0.4	1.8	2%	1	0
24	Amino Acid Metabolism	4	0.009	0.6	1	1.8	1.8	1%	1	0
25	Endocannabinoid Metabolism	4	0.009	0.6	1	1.8	1.7	1%	0	1
26	Amino-Sugar, Galactose, & Non-Glucose	5	0.012	0.7	1	1.4	1.6	1%	1	0
27	Tryptophan, Kynurenine, Serotonin	6	0.014	0.8	1	1.2	1.6	1%	1	0
28	Ceramide Metabolism	34	0.079	4.8	1	0.2	1.5	1%	1	0
								Subtotals	38	23
								Totals	61	

Table S3. Suramin pharmacometabolomics. Metabolites changed at 2-days.

No.	Metabolite	Pathway Name	VIP Score	Z Score	AUC Ratio (Post/Pre)
1	Chenodeoxyglycocholic acid	Bile Salt Metabolism	3.171	1.610	2.787
2	1,25-Dihydroxyvitamin D3	Vitamin D (Calciferol) Metabolism	3.134	-1.447	0.273
3	Glycocholic acid	Bile Salt Metabolism	3.090	2.020	2.344
4	Taurodeoxycholic acid Pool	Bile Salt Metabolism	3.048	1.326	2.614
5	2-Keto-L-gluconate	Microbiome Metabolism	2.994	2.586	1.264
6	Taurocholic acid	Bile Salt Metabolism	2.615	1.102	2.183
7	2,3-Diphosphoglyceric acid	Glycolysis and Gluconeogenesis Metabolism	2.600	0.990	1.198
8	Cytosine	Pyrimidine Metabolism	2.556	2.055	1.689
9	p-Hydroxyphenylacetic acid	Microbiome Metabolism	2.546	1.464	1.192
10	11(R)-HETE	Eicosanoid and Resolvin Metabolism	2.400	-0.875	0.748
11	Hypoxanthine	Purine Metabolism	2.267	-1.000	0.745
12	Deoxyguanosine diphosphate	Purine Metabolism	2.264	-1.276	0.889
13	Glycylproline	Gamma-Glutamyl and other Dipeptides	2.205	1.212	1.773
14	Allantoin	Purine Metabolism	2.195	0.926	1.663
15	L-Isoleucine	Branch Chain Amino Acid Metabolism	2.136	0.815	1.094
16	GC(18:1/22:0)	Glycosphingolipid Metabolism	2.123	1.057	1.399
17	Cysteamine	SAM, SAH, Methionine, Cysteine, Glutathione Metabolism	2.075	1.398	1.107
18	LysoPC(16:0)	Phospholipid Metabolism	2.067	-0.908	0.777
19	Taurine	Taurine, Hypotaurine Metabolism	2.042	-0.942	0.786
20	1-Methyladenine	Purine Metabolism	2.033	1.337	1.631
21	SM(d18:1/20:1)	Sphingomyelin Metabolism	2.033	-1.250	0.745
22	PA(16:0/16:1)	Phospholipid Metabolism	1.998	-0.813	0.793
23	Cyclic adenosine monophosphate	Purine Metabolism	1.949	-0.681	0.855
24	Azelaic acid	Nitric Oxide, Superoxide, Peroxide Metabolism	1.929	-2.024	0.914
25	Shikimate-3-phosphate	Microbiome Metabolism	1.886	1.033	1.047
26	Indoxyl sulfate	Microbiome Metabolism	1.858	0.702	1.280
27	1-Methylhistidine	Histidine, Histamine, Carnosine Metabolism	1.848	0.899	1.145
28	Purine	Purine Metabolism	1.847	1.137	1.203
29	L-Phenylalanine	Tyrosine and Phenylalanine Metabolism	1.839	0.957	1.164
30	Malonic acid	Fatty Acid Oxidation and Synthesis	1.833	-0.825	0.904
31	Methionine sulfoxide	SAM, SAH, Methionine, Cysteine, Glutathione Metabolism	1.817	1.738	1.331
32	L-Valine	Branch Chain Amino Acid Metabolism	1.808	0.749	1.165
33	24,25-Epoxycholesterol	Cholesterol, Cortisol, Non-Gonadal Steroid Metabolism	1.807	1.014	1.362
34	Orotic acid	Pyrimidine Metabolism	1.787	-0.612	0.670
35	AICAR	Purine Metabolism	1.787	1.310	1.309
36	Isovalerylglycine	Branch Chain Amino Acid Metabolism	1.783	0.852	1.951
37	Alanine	Amino Acid Metabolism (not otherwise covered)	1.776	1.066	1.193
38	Xanthosine	Purine Metabolism	1.764	-1.316	0.821
39	Anandamide	Endocannabinoid Metabolism	1.713	-0.709	0.684
40	Citramalic acid	Krebs Cycle	1.704	1.229	1.121
41	Cysteine-S-sulfate	SAM, SAH, Methionine, Cysteine, Glutathione Metabolism	1.682	-0.644	0.869
42	PG(16:0/16:0)	Phospholipid Metabolism	1.664	-0.667	0.549
43	Dopamine	Bioamines and Neurotransmitter Metabolism	1.653	-0.642	0.877
44	Glycerol 3-phosphate	Glycolysis and Gluconeogenesis Metabolism	1.651	1.151	1.187
45	5-HETE	Eicosanoid and Resolvin Metabolism	1.646	-0.671	0.866
46	Myoinositol	Amino-Sugar, Galactose, & Non-Glucose Metabolism	1.645	0.785	1.286
47	L-Glutamic acid	Bioamines and Neurotransmitter Metabolism	1.641	-0.619	0.797
48	Gamma-Aminobutyric acid	GABA, Glutamate, Arginine, Ornithine, Proline Metabolism	1.626	1.101	1.068
49	L-Kynurenine	Tryptophan, Kynurenine, Serotonin, Melatonin Metabolism	1.617	0.625	1.099
50	Citric acid	Krebs Cycle	1.590	0.759	1.142
51	SM(d18:1/20:0)	Sphingomyelin Metabolism	1.576	-0.770	0.712
52	Gamma-glutamyl-Alanine	Gamma-Glutamyl and other Dipeptides	1.575	0.896	1.294
53	Tiglylglycine	Branch Chain Amino Acid Metabolism	1.562	0.657	1.141
54	L-Proline	GABA, Glutamate, Arginine, Ornithine, Proline Metabolism	1.548	0.603	1.155
55	CL(18:2/18:2/18:2/18:2)	Cardiolipin Metabolism	1.538	0.506	1.181
56	CL(18:2/18:2/18:2/18:1)	Cardiolipin Metabolism	1.535	0.474	1.102
57	11,12-Epoxyeicosatrienoic acid	Eicosanoid and Resolvin Metabolism	1.535	-0.634	0.828
58	Ceramide(d18:1/18:2)	Ceramide Metabolism	1.521	0.530	1.337
59	Guanosine	Purine Metabolism	1.519	-0.766	0.702
60	Prostaglandin J2	Eicosanoid and Resolvin Metabolism	1.509	-0.601	0.649
61	N-Acetylglutamic acid	GABA, Glutamate, Arginine, Ornithine, Proline Metabolism	1.505	0.613	1.120

Table S4. Suramin pharmacometabolomics. Metabolites changed at 6-weeks.

No.	Metabolite	Pathway Name	VIP Score	Z Score	AUC Ratio (Post/Pre)
1	2-Keto-L-gluconate	Microbiome Metabolism	2.686	2.365	1.239
2	SM(d18:1/26:0 OH)	Sphingomyelin Metabolism	2.622	2.002	1.671
3	Glycine	1-Carbon, Folate, Formate, Glycine, Serine Metabolism	2.523	1.891	1.392
4	1-Methyladenine	Purine Metabolism	2.459	2.259	2.287
5	Alanine	Amino Acid Metabolism (not otherwise covered)	2.456	1.687	1.322
6	Cytosine	Pyrimidine Metabolism	2.442	2.582	1.932
7	Citric acid	Krebs Cycle	2.410	1.772	1.363
8	1-Pyrroline-5-carboxylic acid	GABA, Glutamate, Arginine, Ornithine, Proline Metabolism	2.358	1.922	1.299
9	Gamma-glutamyl-Alanine	Gamma-Glutamyl and other Dipeptides	2.353	1.725	1.644
10	Histamine	Histidine, Histamine, Carnosine Metabolism	2.279	1.312	1.219
11	p-Hydroxyphenylacetic acid	Microbiome Metabolism	2.203	2.226	1.306
12	Azelaic acid	Nitric Oxide, Superoxide, Peroxide Metabolism	2.151	2.558	1.120
13	Methionine sulfoxide	SAM, SAH, Methionine, Cysteine, Glutathione Metabolism	2.104	2.083	1.409
14	L-Kynurenine	Tryptophan, Kynurenine, Serotonin, Melatonin Metabolism	2.096	1.751	1.303
15	Glycerol 3-phosphate	Glycolysis and Gluconeogenesis Metabolism	2.081	1.731	1.294
16	Cysteamine	SAM, SAH, Methionine, Cysteine, Glutathione Metabolism	2.060	2.007	1.157
17	Chenodeoxyglycocholic acid	Bile Salt Metabolism	2.052	1.650	2.858
18	Hydroxyproline	Vitamin C (Ascorbate) Metabolism	2.039	3.005	1.210
19	2-Hydroxyisovaleric acid	Branch Chain Amino Acid Metabolism	1.988	1.146	1.234
20	Purine	Purine Metabolism	1.980	1.650	1.307
21	Cyclic adenosine monophosphate	Purine Metabolism	1.962	-1.544	0.701
22	Glycocholic acid	Bile Salt Metabolism	1.956	1.945	2.270
23	4-Hydroxyphenyllactic acid	Microbiome Metabolism	1.945	1.172	1.294
24	Deoxyguanosine diphosphate	Purine Metabolism	1.915	-1.583	0.864
25	Hexose Disaccharide Pool	Amino-Sugar, Galactose, & Non-Glucose Metabolism	1.911	1.220	2.121
26	S-Adenosylhomocysteine	SAM, SAH, Methionine, Cysteine, Glutathione Metabolism	1.894	0.971	1.417
27	Isovalerylglycine	Branch Chain Amino Acid Metabolism	1.888	0.901	2.027
28	Allantoin	Purine Metabolism	1.882	1.068	1.798
29	Tiglylglycine	Branch Chain Amino Acid Metabolism	1.878	1.310	1.302
30	L-Phenylalanine	Tyrosine and Phenylalanine Metabolism	1.875	1.381	1.245
31	cis-aconitic acid	Krebs Cycle	1.852	0.928	1.278
32	Lathosterol	Cholesterol, Cortisol, Non-Gonadal Steroid Metabolism	1.844	1.079	1.284
33	L-Asparagine	Amino Acid Metabolism (not otherwise covered)	1.824	1.581	1.360
34	Cinnamoylglycine	Tyrosine and Phenylalanine Metabolism	1.790	2.218	1.190
35	Octanoylcarnitine	Fatty Acid Oxidation and Synthesis	1.780	-1.451	0.703
36	L-Cystine	SAM, SAH, Methionine, Cysteine, Glutathione Metabolism	1.774	1.060	1.190
37	Uridine	Pyrimidine Metabolism	1.764	0.928	1.244
38	Mevalonic acid	Cholesterol, Cortisol, Non-Gonadal Steroid Metabolism	1.673	1.036	1.386
39	Chenodeoxycholic acid	Bile Salt Metabolism	1.670	1.575	2.080
40	Guanidinoacetic acid	SAM, SAH, Methionine, Cysteine, Glutathione Metabolism	1.644	1.254	1.217
41	2-Hydroxyisocaproic acid	Branch Chain Amino Acid Metabolism	1.622	0.997	1.306
42	Decanoylcarnitine	Fatty Acid Oxidation and Synthesis	1.617	-1.157	0.644
43	3-Hydroxy-cis-5-tetradecenoylcarnitine	Fatty Acid Oxidation and Synthesis	1.612	-1.056	0.734
44	Hippuric acid	Microbiome Metabolism	1.559	0.881	1.602
45	PE (18:0/18:0)	Phospholipid Metabolism	1.555	-1.331	0.644
46	L-Proline	GABA, Glutamate, Arginine, Ornithine, Proline Metabolism	1.546	0.749	1.196
47	SM(d18:1/18:2)	Sphingomyelin Metabolism	1.508	0.750	1.424
48	L-Serine	1-Carbon, Folate, Formate, Glycine, Serine Metabolism	1.505	0.954	1.152

Supplemental Data S1. Clinical Global Impression of Improvement questionnaire

Clinical Global Impression

UCSD Suramin Autism Treatment Study

Child's Name: _____

Your Name: _____ (please print)

Date: _____

INSTRUCTIONS

Please answer the following by assessing the full 6-week period after the infusion, compared to your child's behavior before the infusion. If a symptom changed over the 6 weeks, please write in the time after the infusion for maximum change in weeks (wks) or days (d). Please note "wks" for weeks and "days" or "d" for days. For example, if a symptom started to change after 1 week, but didn't reach maximum for 2 weeks, you would write in: "2 wks".
If a symptom didn't change check box "4". If it was never a problem check box "0".

24-Point Autism Symptom Assessment

No.	Over the 6 weeks, how would you assess each of the following?	0 Never a Problem	1 Very Much Improved	2 Much Improved	3 Minimally Improved	4 No Change	5 Minimally Worse	6 Much Worse	7 Very Much Worse	Time after Infusion for maximum change (wks or days) Write-In
1	Overall symptoms of autism severity or delayed development?	☐	☐	☐	☐	☐	☐	☐	☐	
2	Receptive language?	☐	☐	☐	☐	☐	☐	☐	☐	
3	Expressive language?	☐	☐	☐	☐	☐	☐	☐	☐	
4	Difficulty following verbal commands?	☐	☐	☐	☐	☐	☐	☐	☐	
5	Flapping or self-stimulation?	☐	☐	☐	☐	☐	☐	☐	☐	
6	Sensory issues like problems with touch, texture, taste, smell, sound, light, etc.?	☐	☐	☐	☐	☐	☐	☐	☐	
7	Insistence on sameness or difficulty with transitions?	☐	☐	☐	☐	☐	☐	☐	☐	
8	Anxiety or panic attacks?	☐	☐	☐	☐	☐	☐	☐	☐	
9	Tantrums or Meltdowns?	☐	☐	☐	☐	☐	☐	☐	☐	
10	Obsessive and/or compulsive behaviors?	☐	☐	☐	☐	☐	☐	☐	☐	
11	Self-Injurious behavior?	☐	☐	☐	☐	☐	☐	☐	☐	
12	Outbursts of anger or aggression?	☐	☐	☐	☐	☐	☐	☐	☐	
13	Lack of imaginative, make-believe, or age-appropriate play?	☐	☐	☐	☐	☐	☐	☐	☐	
14	Lack of desire for social interaction?	☐	☐	☐	☐	☐	☐	☐	☐	
15	Hyperactivity?	☐	☐	☐	☐	☐	☐	☐	☐	
16	Lethargy or fatigue?	☐	☐	☐	☐	☐	☐	☐	☐	
17	Inattention?	☐	☐	☐	☐	☐	☐	☐	☐	
18	Lack of eye contact or gaze avoidance?	☐	☐	☐	☐	☐	☐	☐	☐	
19	Problems sleeping?	☐	☐	☐	☐	☐	☐	☐	☐	
20	Sound sensitivity or ear covering?	☐	☐	☐	☐	☐	☐	☐	☐	
21	Feeding problems?	☐	☐	☐	☐	☐	☐	☐	☐	
22	Gross motor problems like trouble with abnormal walking or running?	☐	☐	☐	☐	☐	☐	☐	☐	
23	Fine motor problems like trouble with buttons, zippers, snaps, or tripod grasp?	☐	☐	☐	☐	☐	☐	☐	☐	
24	Problems with bowel movements?	☐	☐	☐	☐	☐	☐	☐	☐	

Comments and recommendations:

Page 1 of 2

INSTRUCTIONS

Write down the 3 symptoms that changed the most during the 6 weeks after infusion

Child's Name: _____

Date: _____

No. Over the 6 weeks, what 3 symptoms changed the most?

		1 Very Much Improved	2 Much Improved	3 Minimally Improved	4 No Change	5 Minimally Worse	6 Much Worse	7 Very Much Worse	Write-In: Time after Infusion for maximum change (wks or days)
1		☐	☐	☐	☐	☐	☐	☐	☐
2		☐	☐	☐	☐	☐	☐	☐	☐
3		☐	☐	☐	☐	☐	☐	☐	☐

Supplemental Data S2. Social Stories to Accompany the Storyboard Panels Describing Each Step of the Infusion Day Visit.

Check-in. "Hello again! You and your mom or dad are at our clinic today! We will do lots of different things, and meet different people. Everybody here is really nice. First, you will check in at the front desk, to let the doctor and nurses know that you are here. You might have to wait a few minutes before the nurse gets you. That's okay. You can sit in a chair and play with any toys that you brought with your today."

Numbing Medicine. "Then you will meet the nurse. She is really nice and friendly. You will sit in a chair or on the bed, and the nurse will put a special medicine on your arms, on the inside of your elbows (right where it bends.) The medicine will make your arms tingly and numb, and might tickle a little. That's okay, that's how we know that the medicine is working."

Height and Weight. "The nurse will take you to another room. You will stand on a scale and measure your weight, and you will stand tall to measure how tall you are. The nurse will also measure your blood pressure with a special bracelet that goes around your arm. She will take your temperature by touching your forehead with a fast thermometer."

Urine Sample. "If you didn't pee in a cup at home before you came to the clinic today, you will pee in a cup at the doctor's office in the bathroom. Mom or Dad will go with you if you need help."

Blood Sample. "After the bathroom, you will see the nurse again. Your arm will be nice and numb. The nurse will put a special needle in your arm, take some blood, then take out the needle and leave in a little plastic tube called an IV. Great job! That didn't hurt too much, and you sat so nice and still! The nurse will take some blood out of the tube, put some medicine in the tube, then wrap up your arm so you can go and play! We have lots of toys to play with. Or you can plan with the toys that you brought with you."

IV. "After some play time, you will sit down or lay down quietly, with no walking or jumping. A long tube called and IV will put medicine into the little tube in your arm. You can watch TV or play with your iPad, or even some Legos. Mom or Dad will sit with you the whole time."

Post-Infusion Free Time. "Next, the big tube gets put away, your arm gets wrapped up again, and you get to play some more! Or watch more TV. Have fun with your mom or dad."

Thank You Gift. "The nurse will then take the little tube out of your arm. Then you are done! Great job! You get to pick a present or have a treat, then go home with Mom or Dad. Thank you for being such a good helper today, and sitting so nicely and quietly. You had a good quiet mouth and gentle hands, and that makes Mom and Dad so happy. You did great!"

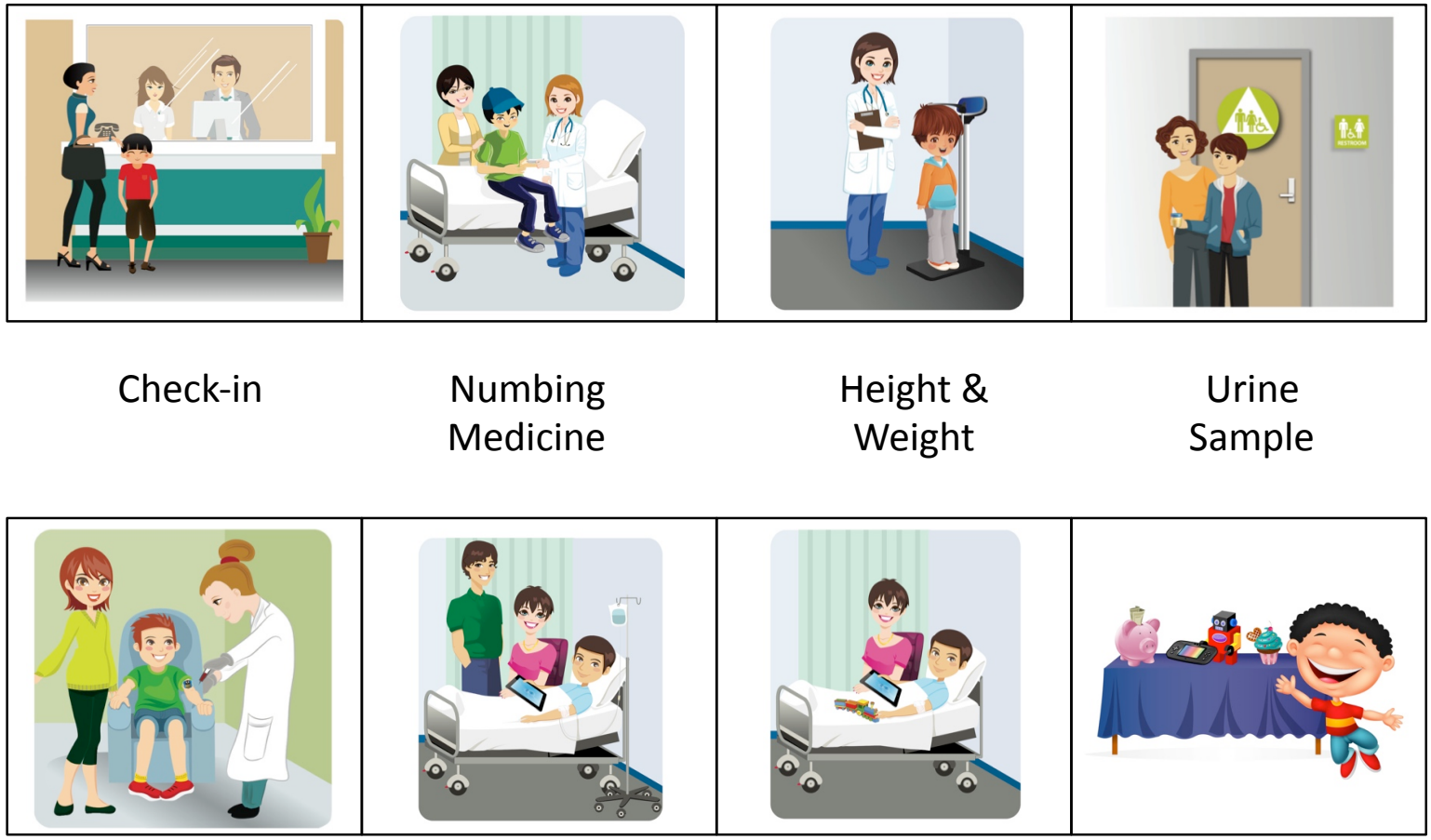


FIGURE S1
Storyboard illustration of each step of the infusion day visit

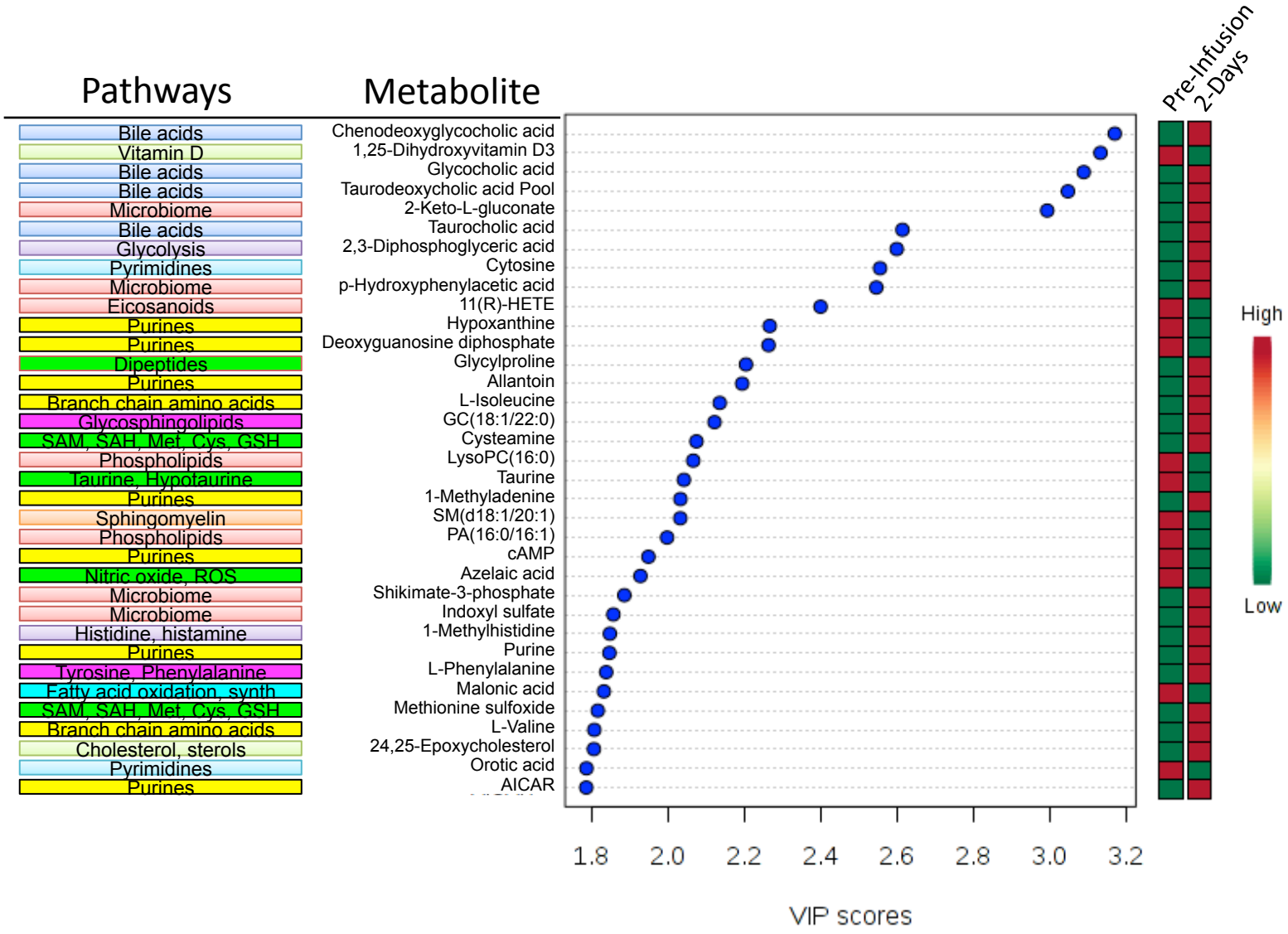
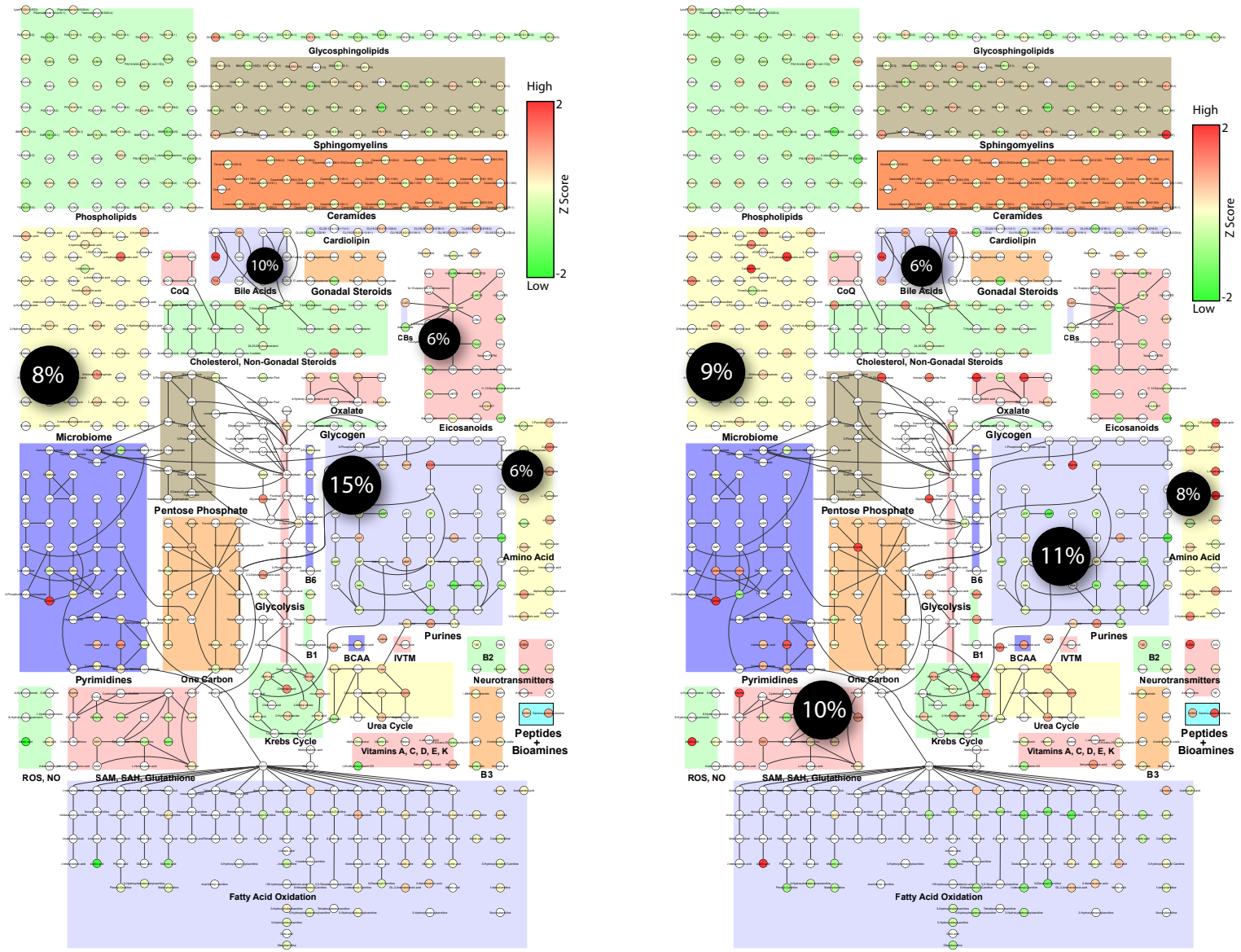


FIGURE S2

Suramin pharmacometabolomics. Metabolites and pathways changed at 2 days



2-Days 6-Weeks

FIGURE S3. Suramin pharmacometabolomics. Pathway visualization.

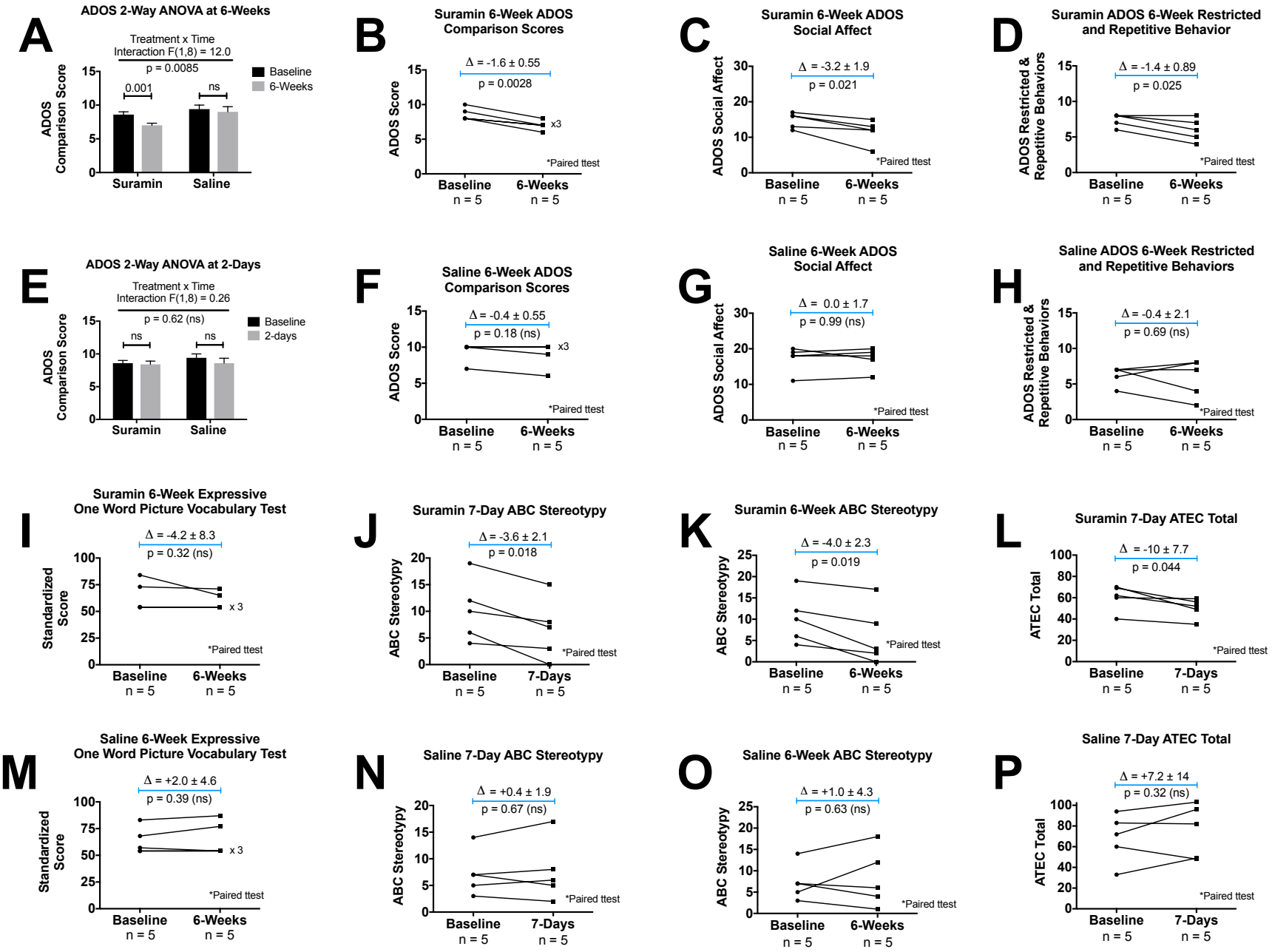


FIGURE S4 Outcomes, A-P

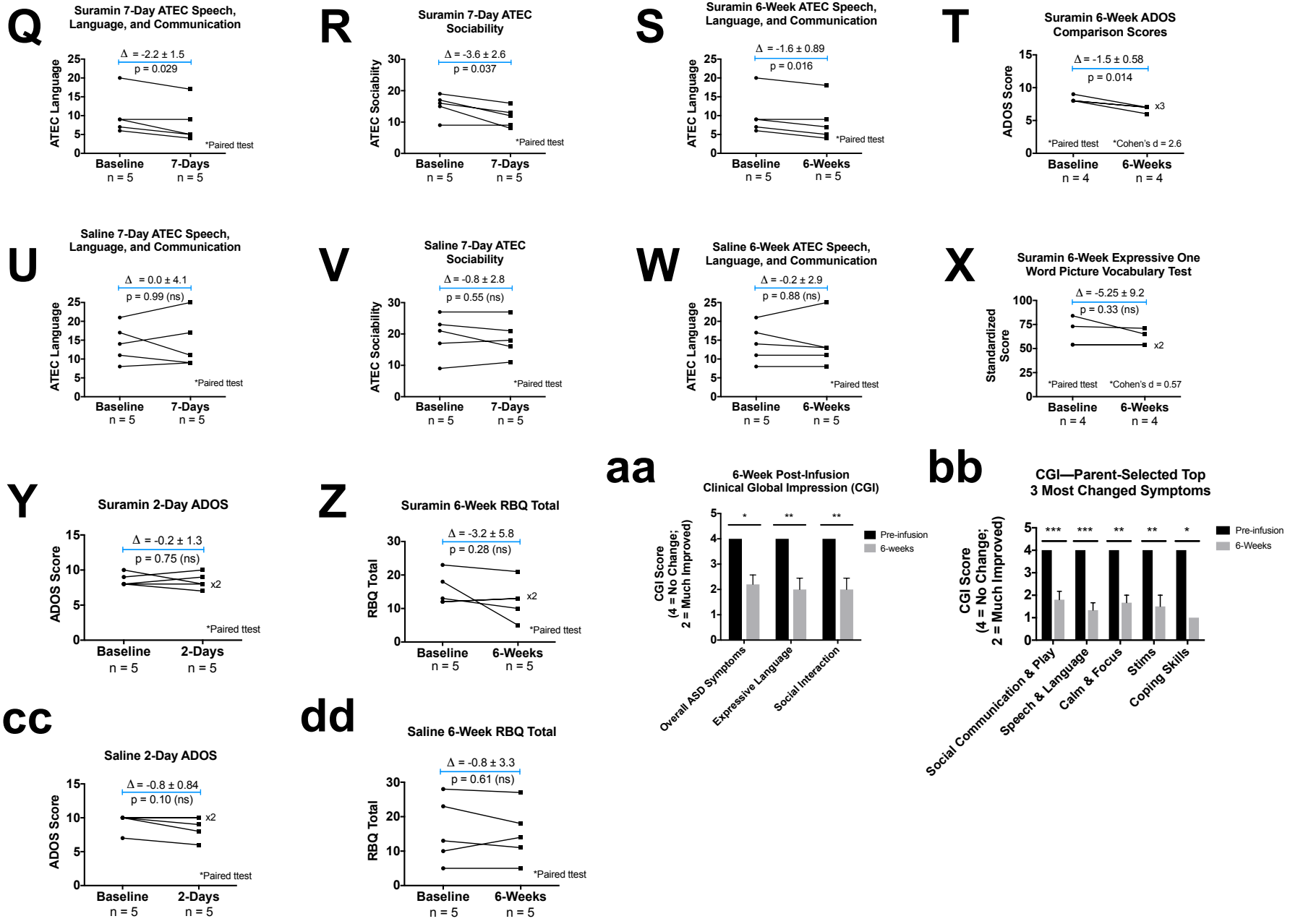


FIGURE S4 Outcomes, Q-dd