Supplementary File 2

Methods:

Microbiome Culture test

Culture tests were conducted to investigate specific *Clostridium* species (*C. difficile, C. bolteae, C. perfringens* and *Hungatella hathewayi/effluvia*, previously known at *Clostridium histolyticum*) and yeast, which have all been reported to be elevated in children with ASD (Alshammari et al., 2020; Kandeel et al., 2020; Nirmalkar et al., 2024) and hence were suspect as being elevated in children with PTHS. Also, a previous study had found a higher abundance of *C. bolteae* in children with PTHS (Dilmore et al., 2021). Fecal samples were sent to Doctors Data, USA (a CLIA-approved laboratory) for their anaerobic *Clostridium* and yeast culture tests.

Results:

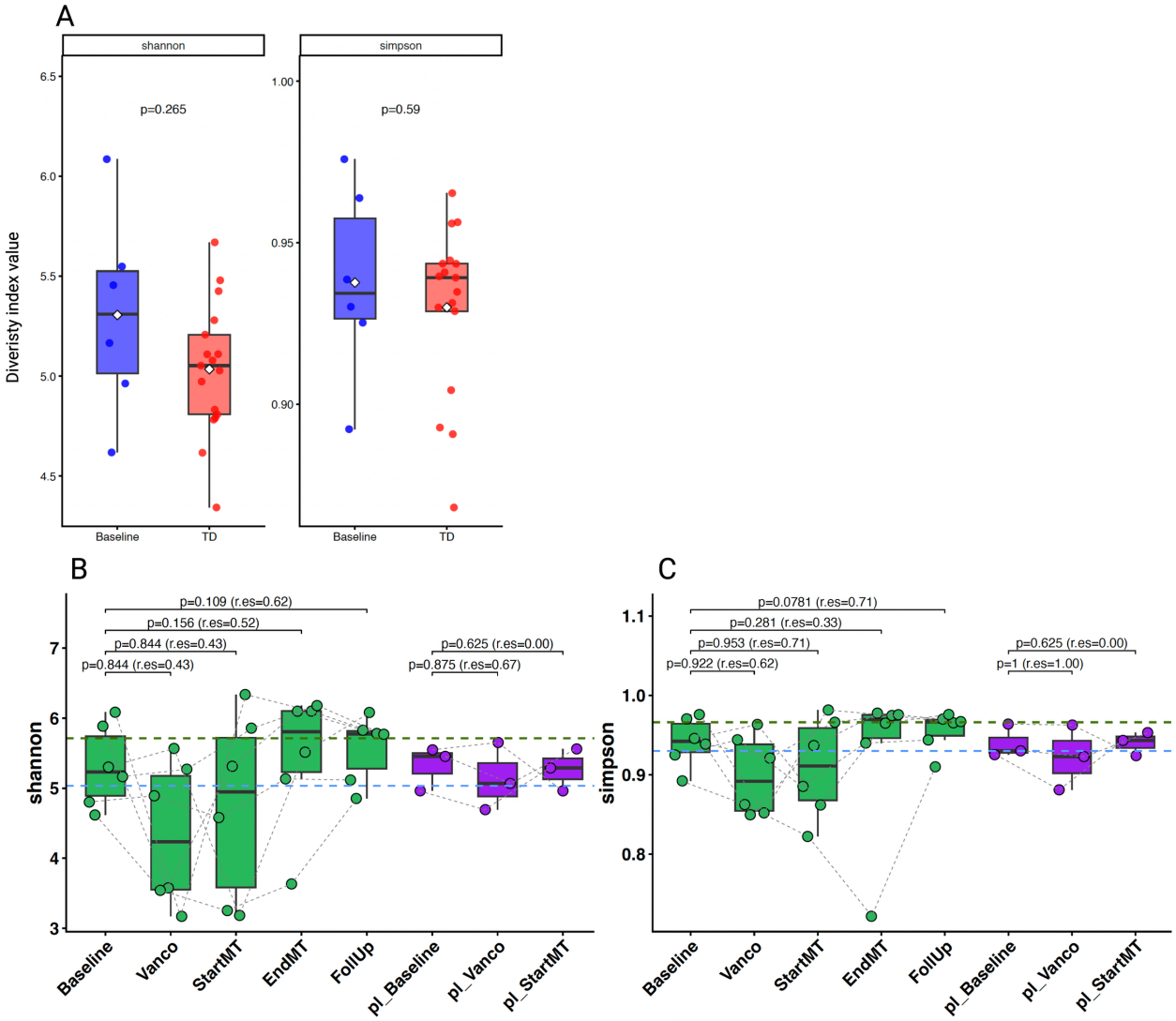

Figure S4: Alpha diversity for baseline and typically developing; A) Shannon and Simpson, and before and after MTT in children with PTHS in; (B)Simpson, (C) Shannon diversity index. Each colored dot represents a study participant. horizontal green shows the mean relative abundance of

donor and blue line for the typically developing (TD) controls. MT = Microbiota Transplant; Vanco = vancomycin; StartMTT = start of Microbiota Transplant; EndMT = end of Microbiota Transplant; FollUp = Follow-up; pl = placebo; es= Wilcoxon effect size. All p-values <0.05 (unadjusted) were considered statistically significant.

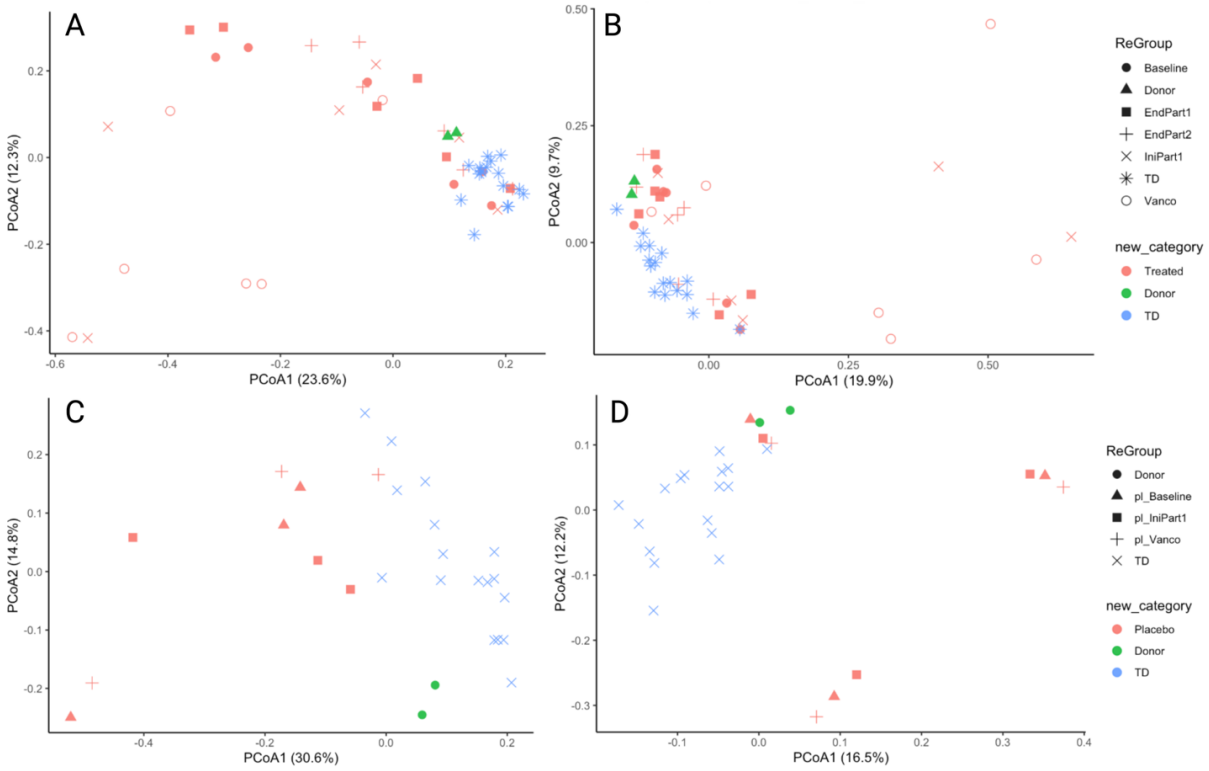

Figure S5: Beta diversity before and after MTT in children with PTHS using principal coordinate analysis (PCoA). The Bray-Curtis (A); Jaccard (B) for treatment group; Bray-Curtis (A); Jaccard (B) for placebo group. Each colored dot represents a study participant. MTT=Microbiota Transplant Therapy; Vanco=vancomycin; IniMTT=start of MTT treatment; EndMTT=End of MTT treatment; pl=placebo.

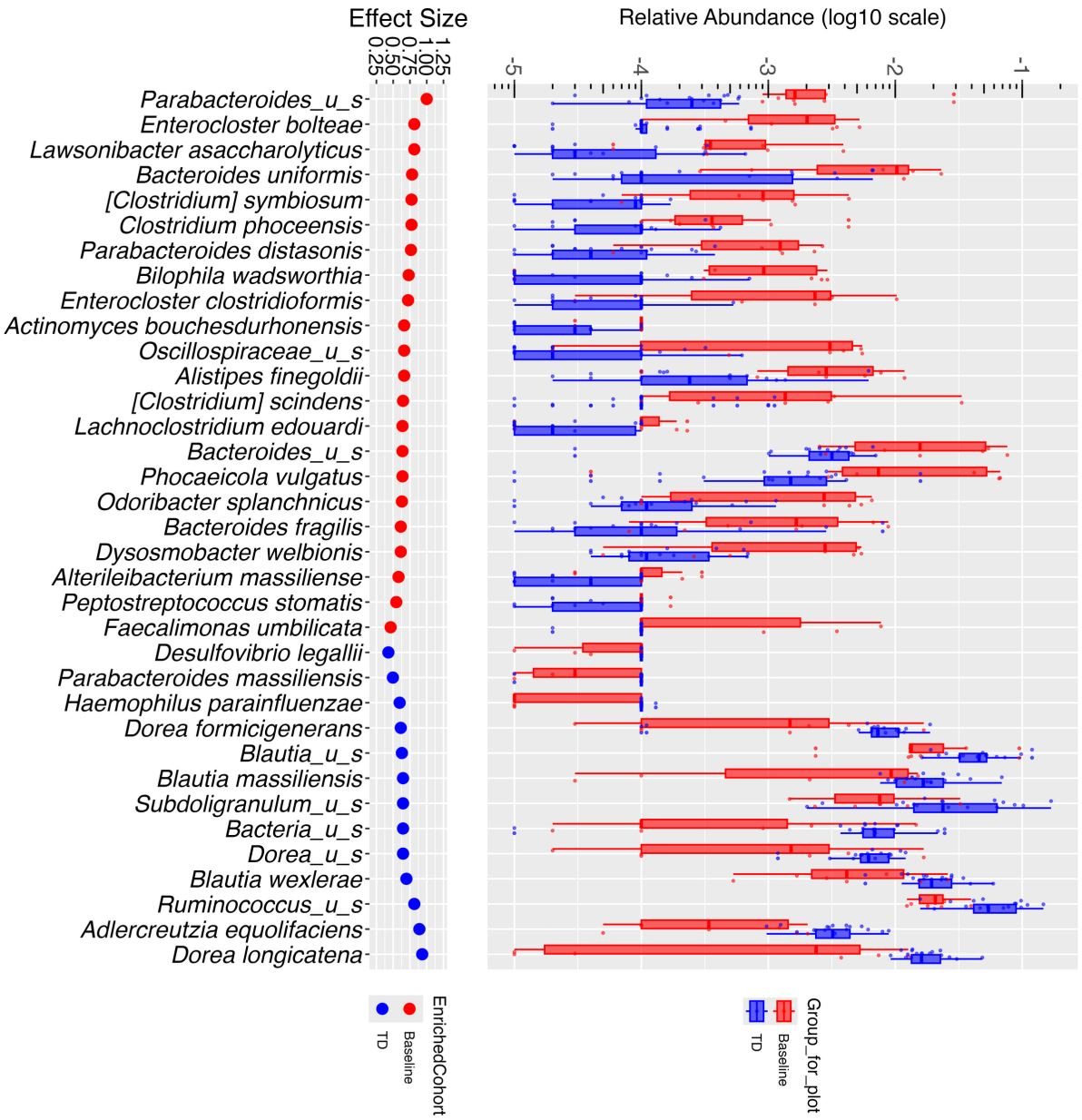

Figure S6. Differences in microbiota composition between children with PTHS (red) at baseline and typically developing (TD) (blue) using shotgun metagenomics data. Effect sizes (left panel) shows the magnitude and direction of group differences, with red for higher in ASD, and blue for higher in TD. For baseline comparisons, all available PTHS baseline samples (n = 7), including those from Group-A (n = 3), Group-B (n = 3), and one participant who later withdrew, were analyzed. Horizontal boxplots (right panel) display taxa that differed significantly in relative abundance (log_{10}-transformed). Plot shows the top 35 significantly different taxa, See supplementary file S3 for the complete list of taxa. Statistical comparisons were performed using one-tailed Mann-Whitney U tests ($p<0.05$). Due to the ultra-rare nature of the disorder and limited sample size, FDR correction was not applied. See the Statistical Analysis section for details.

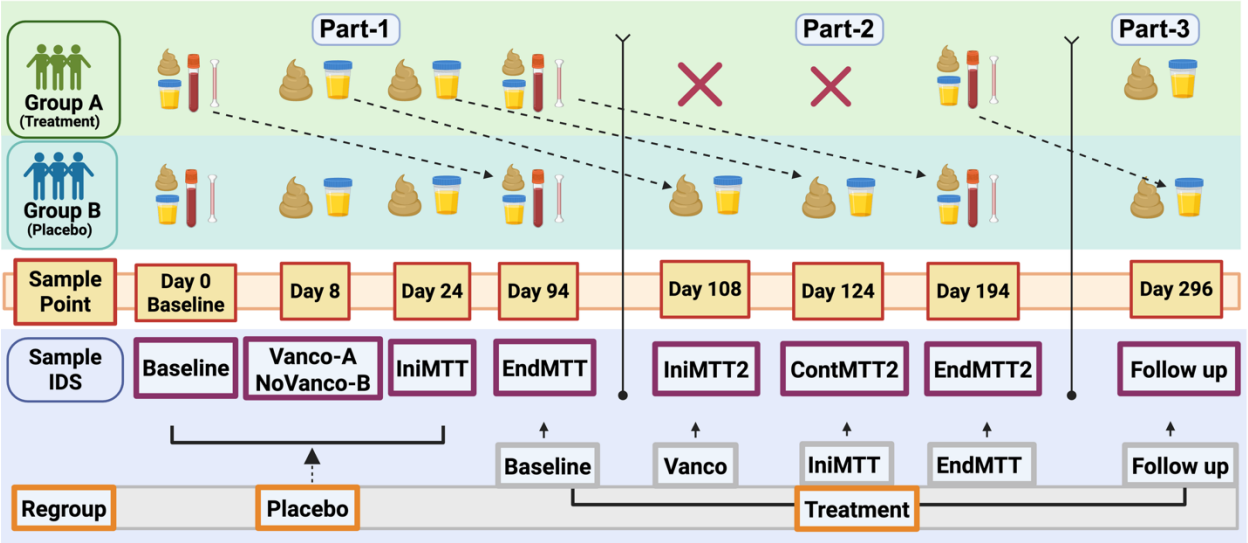

Figure S7: Regrouping the study time points for longitudinal microbiome data analysis. Blue color for TD and red color for children with PTHS at baseline. MTT- microbiota transplant therapy; Vanco-vancomycin; IniMTT- start of the MTT/treatment; EndMTT- end of the MTT/treatment; ContMTT2-continuation of the MTT/treatment in Part-2; NoVanco- without vancomycin; Follow up- 14 weeks of follow up. See statistical analysis of Methods section for more details.

***Initial findings from 16S data*:**

Alpha diversity analysis from 16S rRNA gene amplicon sequencing data showed no substantial change after MTT compared to baseline in either treatment (group-AB) or placebo (group-B, Part1) (Supplementary Figure S6A-B). Beta diversity, weighted UniFrac distance-to-donor (a dissimilarity metric for abundant taxa with phylogenetic distance) did not change significantly after treatment compared to baseline (Supplementary Figure S6C) in either group. In contrast, Jaccard distance-to-donor (a presence/absence-based metric accounting for lower abundant taxa) significantly decreased after treatment compared to baseline in treatment (group-AB) and became more similar to the donor. No significant change was observed in placebo (group-B, Part1) (Supplementary Figure S6D).

Similar to alpha diversity, the relative abundance of gut microbial taxa showed no significant change after MTT compared to baseline in either treatment (group-AB) or placebo (group-B, Part1) (Supplementary Figure S7).

We observed changes in the relative abundance of specific beneficial microbes such as *Faecalibacterium* species (Supplementary Figure S5A), which significantly decreased ($p<0.05$) at vancomycin pretreatment compared to baseline in the treatment (group-AB). Following MTT, *Faecalibacterium* (both end of MTT and after 3-months of MTT) significantly increased ($p<0.05$) compared to the vancomycin group. A similar trend was observed (vancomycin compared to end of MTT or after 3-months of MTT timepoints) for *Bacteroides vulgatus*, *Blautia* and *Romboustia* (Supplementary Figure S7B-D). This result suggests that MTT may help to support the re-establishment of certain microbes after vancomycin pretreatment depletion.

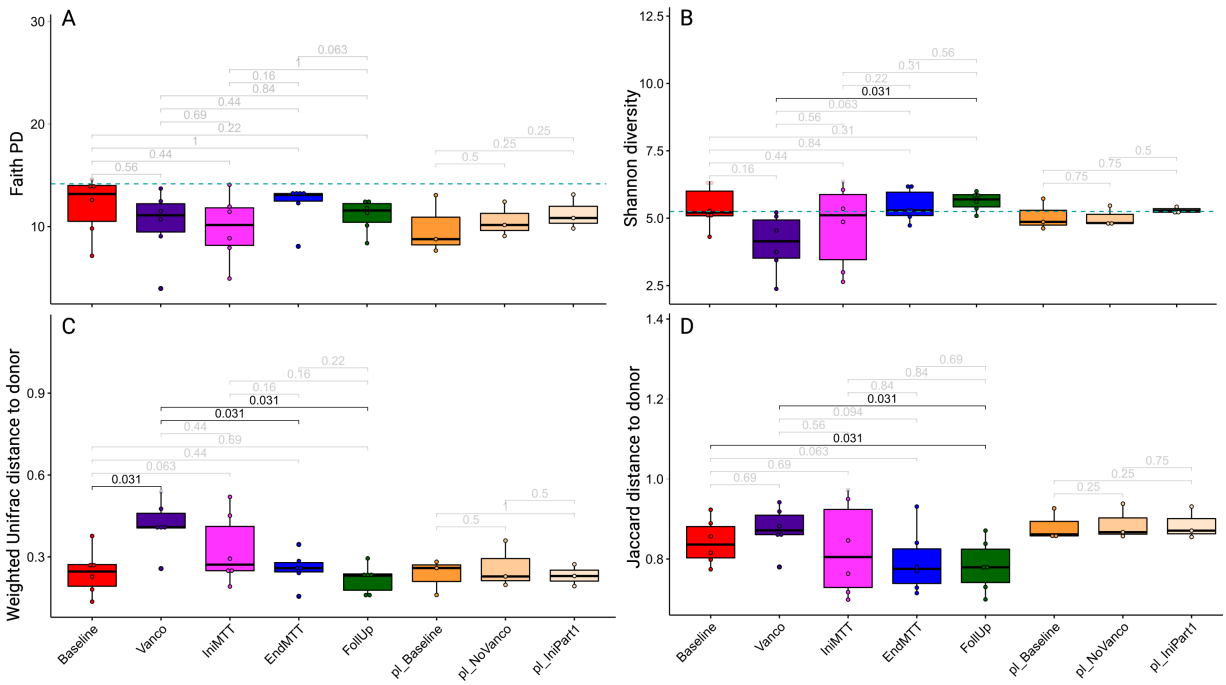

Figure S8: Alpha diversity and distance-to-donor using beta-diversity before and after MTT in children with PTHS.

The Faith PD (A); Shannon diversity index (B); Weighted Unifrac distance-to-donor (C); Jaccard distance-to-donor. Each colored dot represents a study participant. Light blue dashed lines represent the mean of the donor (n=2). All p-values <0.05 (unadjusted) were considered statistically significant.

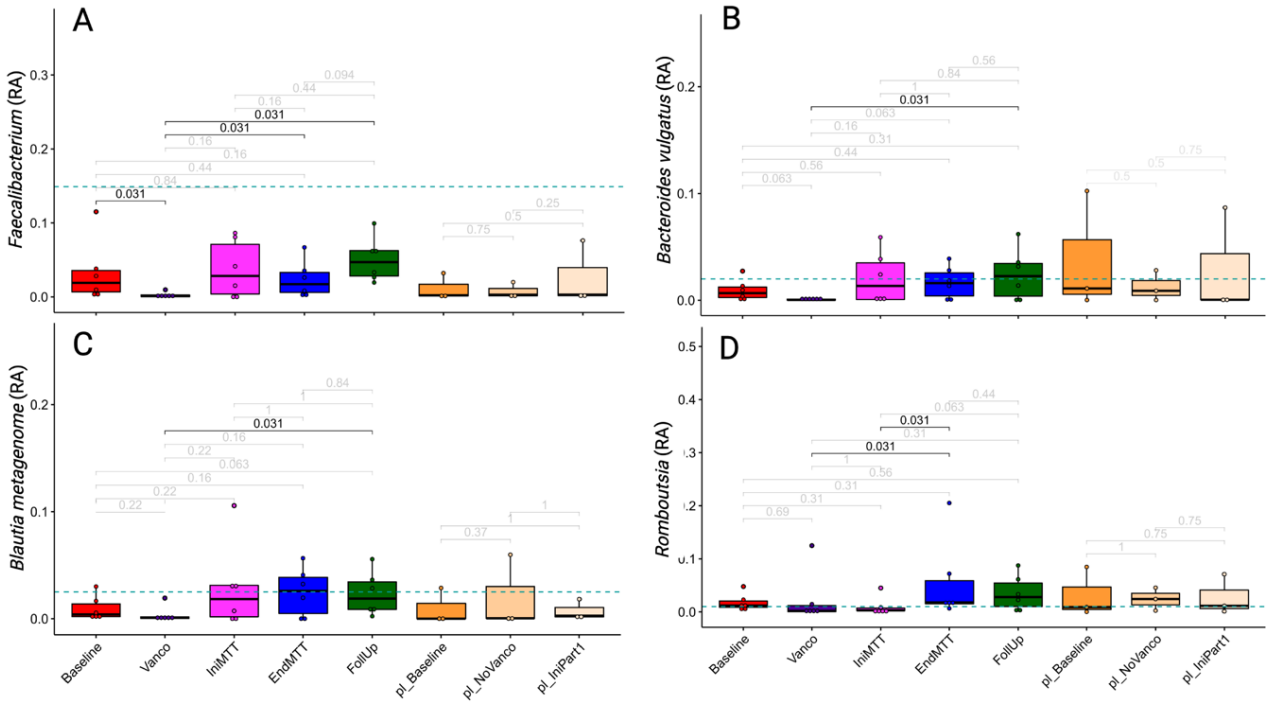

Figure S9 Relative abundance of 16S rRNA gene amplicon sequences of specific gut microbiota before and after MTT in children with PTHS. A) *Faecalbacterium,* B) *Bacteroides*, C) *Blautia metagenome*, D) *Romboutsia* shows the longitudinal comparison between groups in treatment and placebo group. Horizontal blue line shows the mean relative abundance of donor. MTT=Microbiota Transfer Therapy; Vanco=vancomycin; IniMTT=start of MTT treatment; EndMTT=End of MTT treatment; pl=placebo. Paired-wise Wilcoxon sign-rank test was applied for longitudinal comparison and $p<0.05$ was considered as statistically significant. Due to small sample size, p-values were not corrected for multiple hypothesis test (false discovery rate).

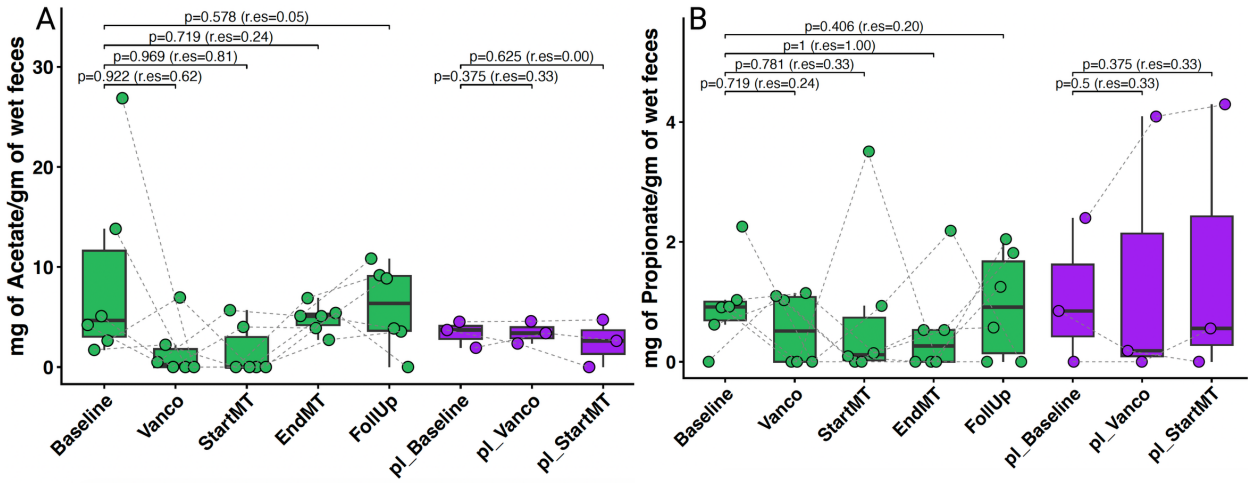

Figure S10: Fecal short chain fatty acid measurements A) **Acetate**, B) **Propionate** (in per gram of wet feces). MT = Microbiota Transplant; Vanco = vancomycin; StartMTT = start of Microbiota

Transplant; EndMT = end of Microbiota Transplant; FollUp = Follow-up; pl = placebo; Wilcoxon sign-rank test was applied for longitudinal comparison and $p<0.05$ was considered as statistically significant.

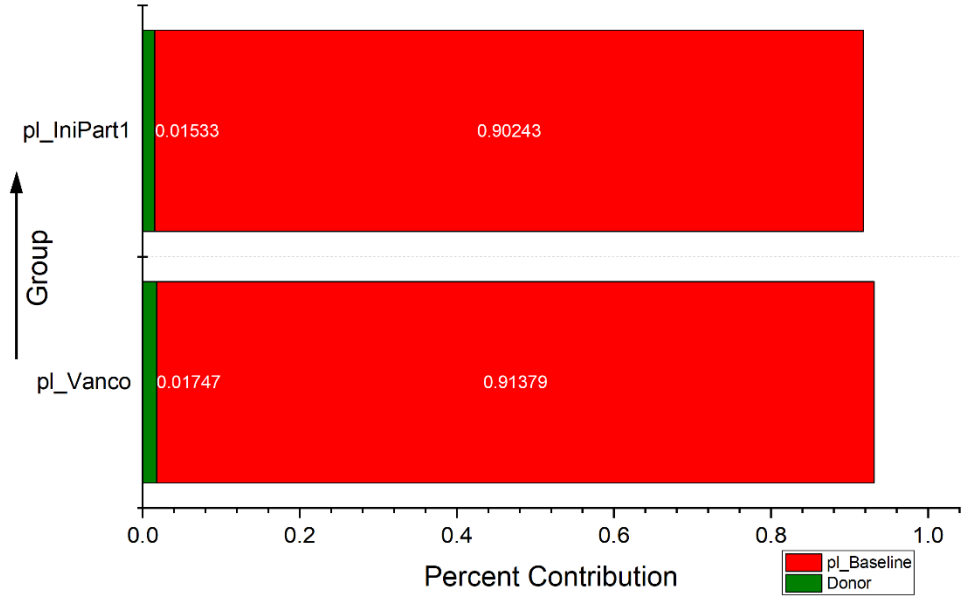

Figure S11: SourceTracking analysis in the group-B while on placebo. Estimated contribution of baseline microbiota (pl_Baseline, red color) and donor (green color) microbiota for the group-B while on placebo after 8 days of placebo vancomycin (pl_Vanco) and after 14 days of placebo microbiota (pl-IniPart1). The x-axis shows the percent contribution of ASVs and the y-axis for the placebo time points.

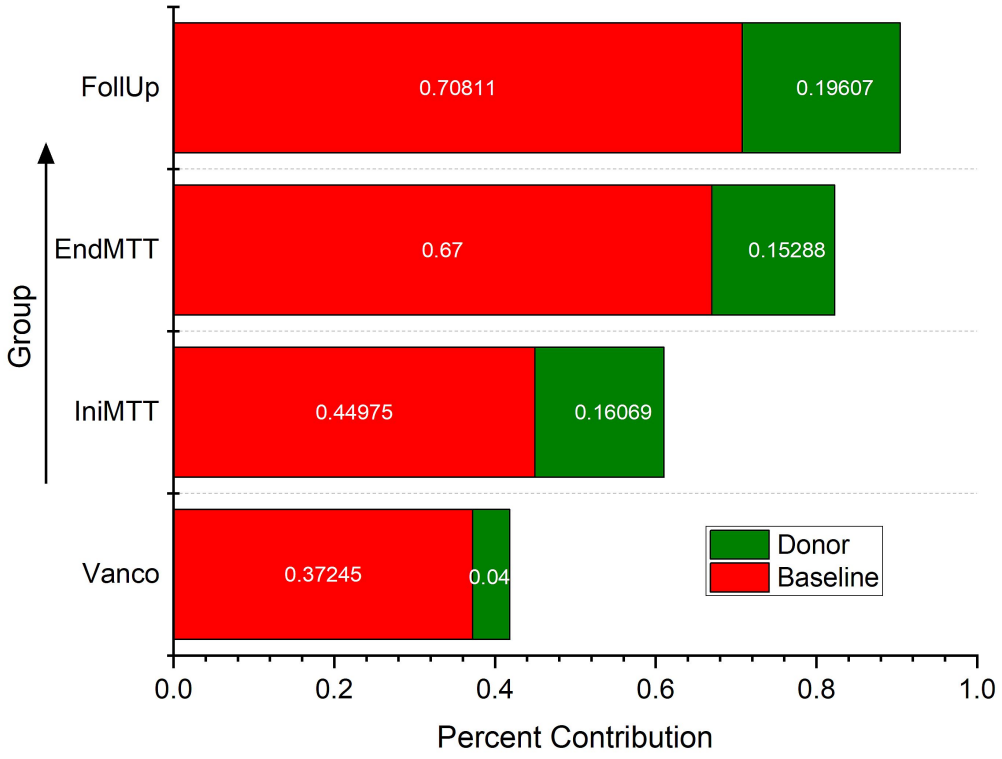

Figure S12: SourceTracking analysis in the treatment group. Estimated contribution of baseline microbiota (Baseline, red color) and donor (green color) microbiota for the group-A after 8 days of vancomycin (Vanco), after 14 days of microbiota treatment (IniPart1), after 10 weeks (EndMTT), and after 3 months of treatment (FollUp). The x-axis shows the percent contribution of ASVs and the y-axis for the treatment time points.

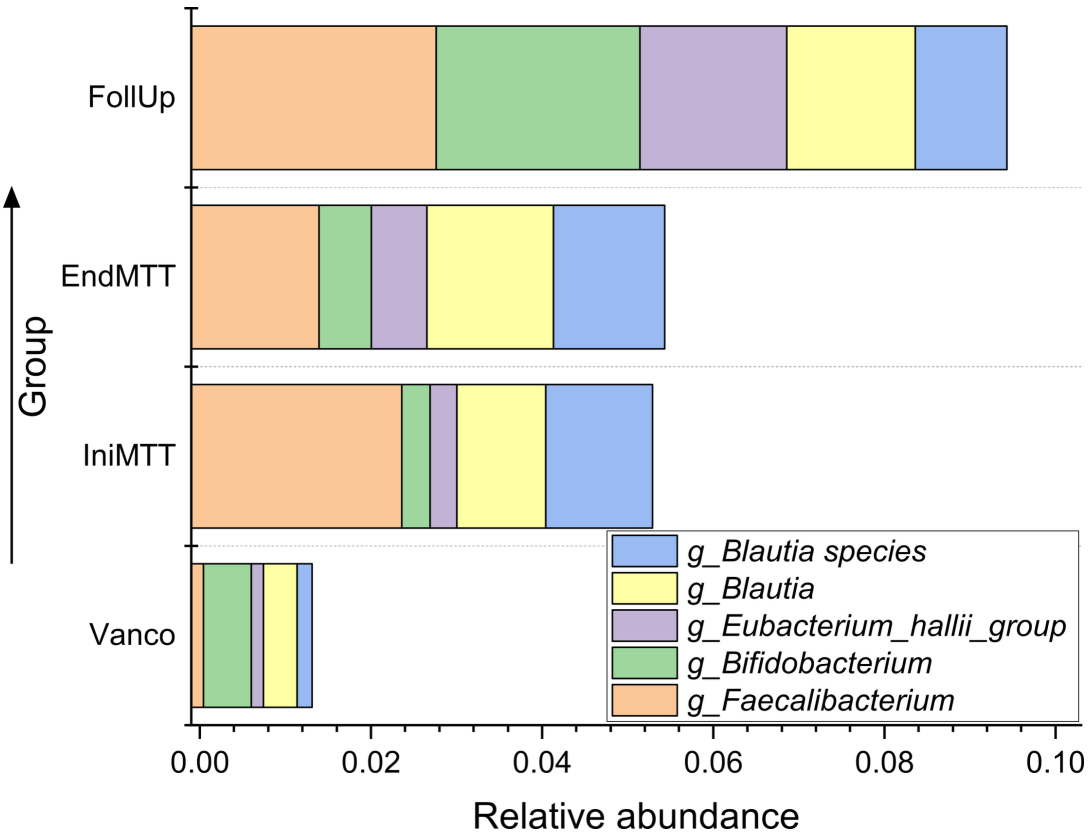

Figure S13: The top five bacteria which primarily contributed to the % engraftment in the treatment group (from Figure S12), at the different time points. The x-axis shows the percent relative abundance of ASVs and the y-axis for the treatment time points. Overall, the variation in % engraftment may suggest that higher and/or longer dosages or booster dosages may be beneficial for some individuals to increase and/or maintain % engraftment. This suggests that higher and/or extended dosages may result in greater symptom benefit in some cases.

MetaCyc Genes for metabolic pathways differ between PTHS and TD, but do not significantly change after MTT

Microbial functional gene abundance was analyzed to study metabolic pathways using MetaCyc data derived from shotgun metagenomics. Comparing the relative microbial gene abundance between baseline and TD controls revealed 66 significantly different MetaCyc pathways ($p < 0.05$, $q < 0.1$) with large effect sizes (Supplementary Figure S14). Of these, 44 pathways were significantly higher and 22 significantly lower at baseline compared to TD.

Most of the significantly elevated pathways at baseline were related to amino acid metabolism (e.g., *L-histidine biosynthesis*, *GABA shunt*), cofactor and vitamin metabolism (e.g., *superpathway of heme b biosynthesis from glutamate*, *biotin biosynthesis I*), lipid metabolism (e.g., *lipid IVA biosynthesis [E. coli]*, *fatty acid elongation -saturated*), and nucleotide metabolism (e.g., *superpathway of adenosine nucleotides de novo biosynthesis I*, *adenosine deoxyribonucleotides de novo biosynthesis II*). In contrast, pathways that were significantly lower at baseline compared to TD were primarily associated with carbohydrate metabolism (e.g.,

sucrose biosynthesis II, *glycogen degradation*) and cofactor and vitamin metabolism (e.g., *superpathway of tetrahydrofolate biosynthesis*, *molybdopterin biosynthesis*).

When the relative abundance of genes from MetaCyc pathways was compared from baseline to post-treatment timepoints, no significant changes ($p < 0.05$, $q < 0.1$) were observed in either the treatment (group-AB) or placebo (group-B, Part1) groups.

EC Genes for metabolic pathways differ between PTHS and TD, significantly change after MTT

Comparison of the relative abundance of microbial EC genes encoding enzymes across various pathways between the baseline and TD controls revealed 130 significantly different EC genes ($p < 0.05$, $q < 0.1$) with large effect sizes (Supplementary Figure S15). Of these, 54 EC genes were significantly higher and 76 significantly lower at baseline compared to TD.

Top 30 most significantly different EC showed that *Glutamate dehydrogenase (EC 1.4.1.2)* and *histidine ammonia-lyase (EC 4.3.1.3)* were higher at baseline, suggesting shifts in microbial glutamate and histidine metabolism pathways closely linked to excitatory neurotransmission and histamine signaling. In contrast, lower abundance of *glutamate synthase (NADPH) (EC 1.4.1.13)* and *glycine reductase (EC 1.21.4.2)* may reflect disruptions in glutamate-glutamine cycling and microbial glycine processing, both of which are relevant to synaptic regulation and NMDA (N-methyl-D-aspartate- a specific receptor for glutamate and ion channel) receptor activity. A higher abundance of *lipoyl synthase (EC 2.8.1.8)* suggests enhanced microbial production of lipoic acid, a mitochondrial cofactor that supports oxidative metabolism and has been linked to neurodevelopmental function. Meanwhile, a lower abundance in lysozyme (EC 3.2.1.17), a host-associated antimicrobial enzyme, may indicate compromised innate defense and altered host–microbiome interactions. Together, these microbial EC gene abundance shifts suggest altered neuroactive and immunomodulatory functions that could contribute to both GI and neurological symptoms observed in PTHS.

When the relative abundance of genes from EC pathways was compared from baseline to post-treatment timepoints, no significant changes were observed in either treatment (group-AB) or placebo (group-B, Part1) groups. Larger sample sizes may be needed to detect significant changes.

AMR genes differ between PTHS and TD but do no significant change after MTT

Using shotgun metagenomics, we also calculated the relative abundance of antimicrobial resistance (AMR) genes and identified 7 AMR genes that were significantly higher ($p<0.05$) with a large effect size at baseline compared to TD. These ARG genes belonged to three different types of antibiotic categories; tetracycline, marcrolide (*macrolide 44 1727 branch, marcrolide 79 1741 branch, macrolide gene msr(D) 3 AF227520, macrolide 92 1836 branch*), and aminoglycoside (*aminoglycoside gene ant(6)-lb 1 FN594949, aminoglycoside gene ant(6)-la 1 AF330699*) (Supplementary Figure S16). When the relative abundance of AMR genes was compared from baseline to after the treatment timepoints, no significant changes were observed in either treatment (group-AB) or placebo (group-B, Part1) groups. While these findings at baseline are intriguing, the lack of statistical significance after MTT may reflect the limited sample size and warrants further validation in larger cohorts.

Figure S14. Differences in functional genes composition from MetaCyc pathways database between children with PTHS at baseline and typically developing using shotgun metagenomic data. Horizontal box plot shows the statistically significant different functional gene (y-axis) in relative abundance (log10, x-axis) and Cohen's d effect size (x-axis) between PTHS at baseline and TD children. Blue color for TD and red color for children with PTHS at baseline.

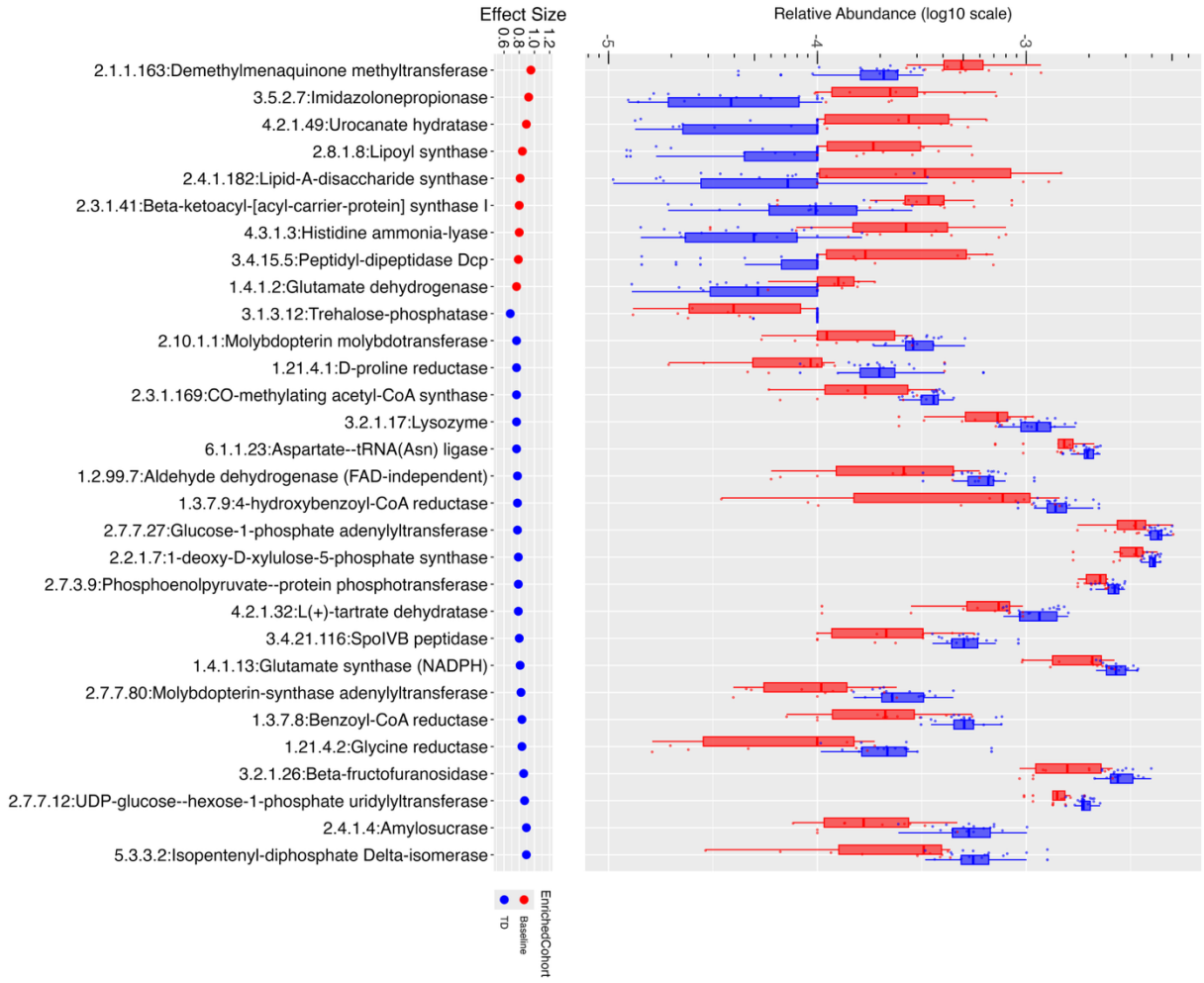

Figure S15: Potential function genes using Enzyme Commission (E.C.) pathway database in PTHS children. Differences in genes from EC pathways between children with PTHS at baseline and typically developing (TD) using shotgun metagenomic data. The horizontal box plot shows the statistically significant different genes (y-axis) in relative abundance (log10, x-axis) and effect size (x-axis) between PTHS at baseline and TD children. Blue color for TD and red color for children with PTHS at baseline. See statistical analysis for the details.

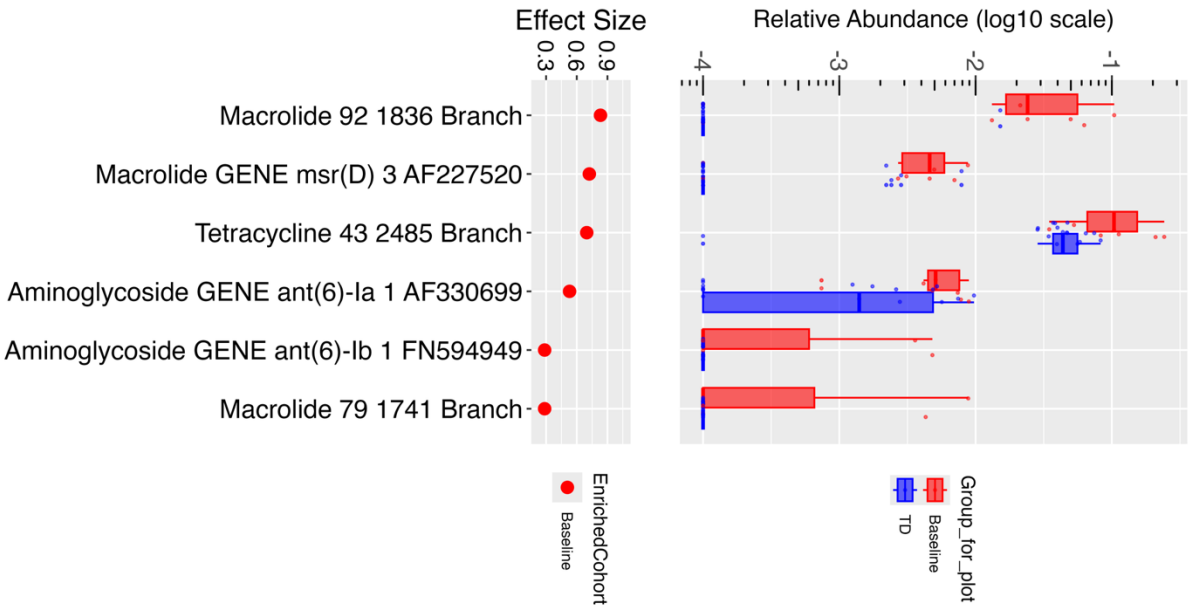

Figure S16. Differences in antimicrobial resistance (AMR) genes relative abundance between children with PTHS at baseline and typically developing. Horizontal box plot shows the statistically significant different taxa (y-axis) in relative abundance (log10, x-axis) and Cohen's *d* effect size (x-axis) between PTHS at baseline and TD children. Blue color for TD and red color for children with PTHS at baseline.

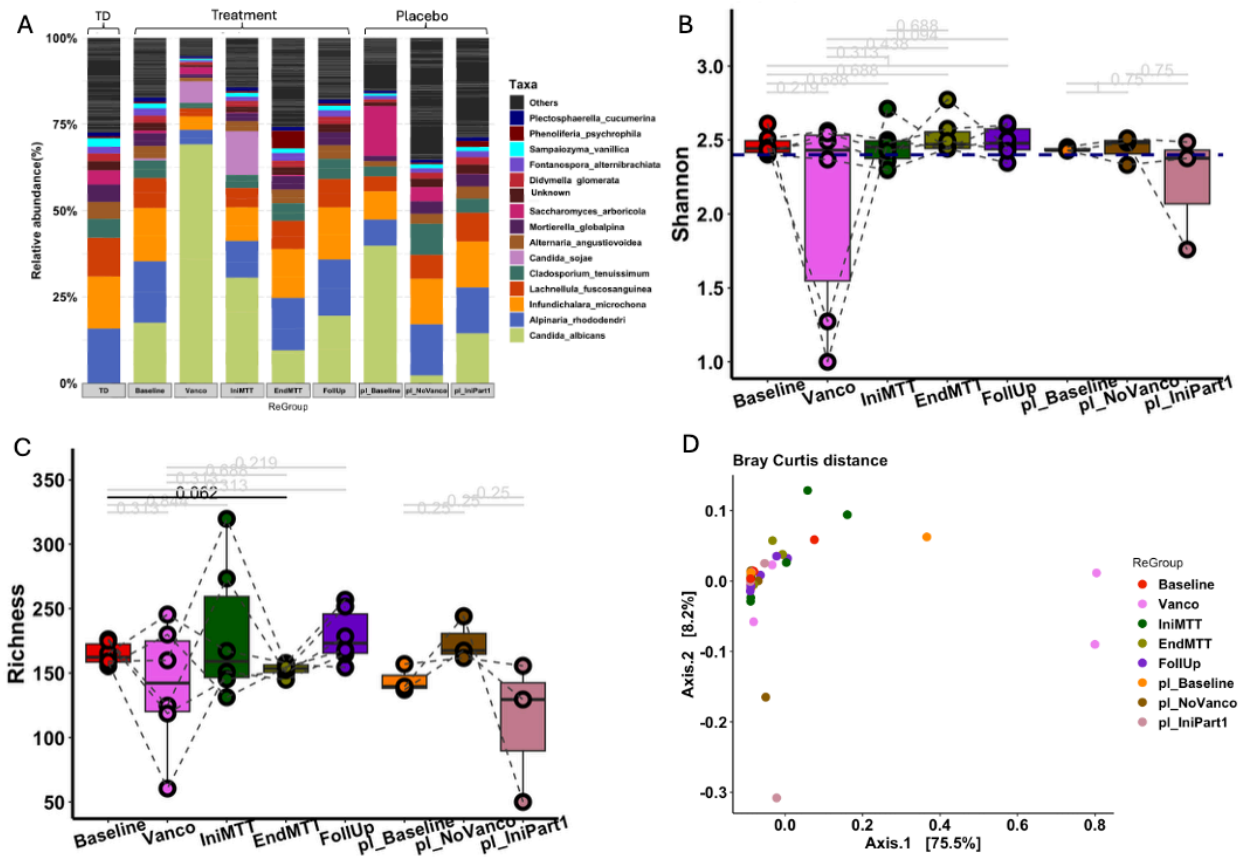

Figure S17: Fungi composition (A) and diversities B) Shannon, C) Richness, D) Bray Curtis distance in children with PTHS before and after MTT using fungal ITS amplicon gene sequencing. Figure-A shows the different groups at X-axis, and y-axis shows the relative abundance (%) of different fungi. MTT=Microbiota Transplant Therapy; Vanco=vancomycin; IniMTT=start of MTT treatment; EndMTT=End of MTT treatment; pl=placebo.

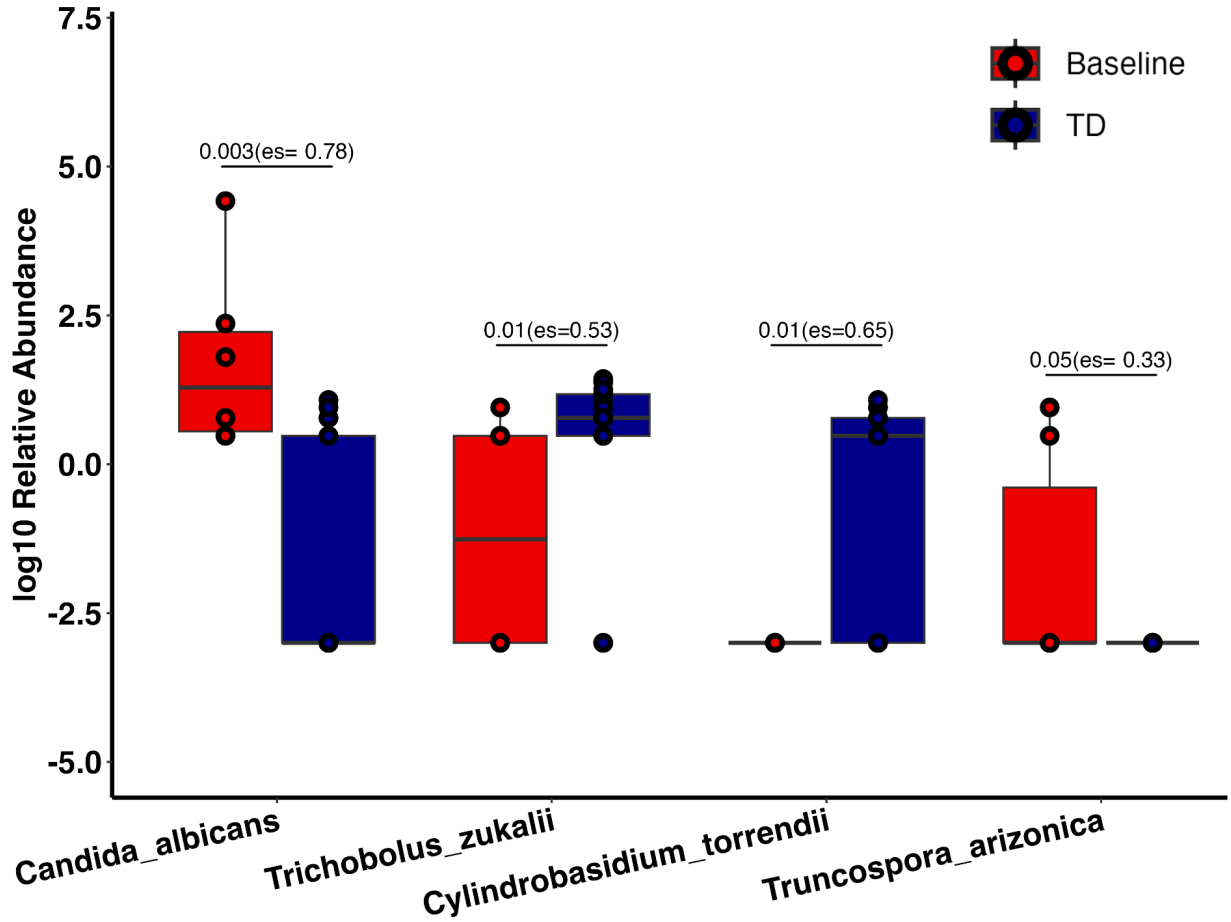

Figure S18: Differences in fungal abundance (log10) between children with PTHS at baseline and typically developing (TD) using fungal ITS amplicon gene sequencing. p-values <0.05 were considered statistically significant and FDR was not applied due to small sample size. Effect size was calculated using Wilcoxon effect size method. es=effect size. See statistical analysis for the details.

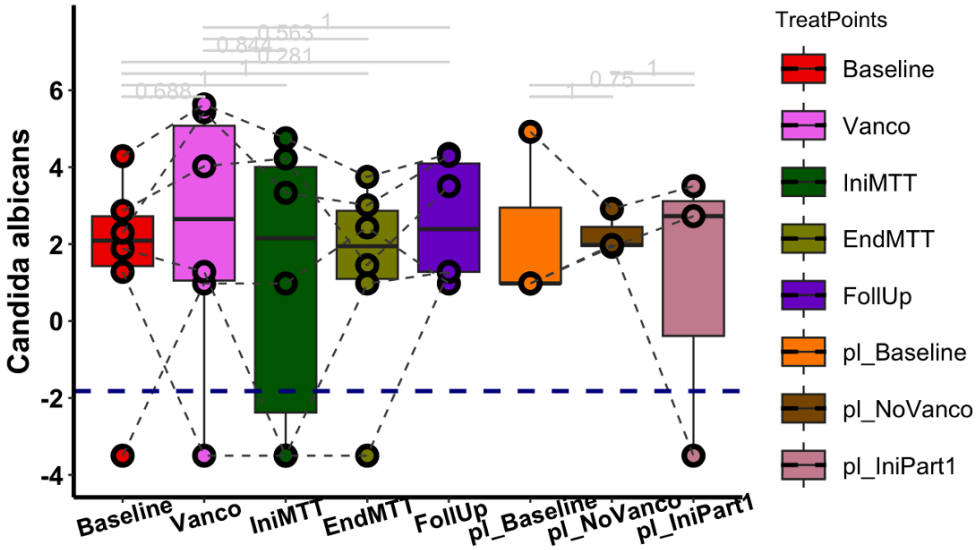

Figure S19: Relative abundance (log10) of *Candida albicans* before and after MTT in children with PTHS. Horizontal dark-blue dashed line shows the mean relative abundance of 17 TD controls. MTT=Microbiota Transplant Therapy; Vanco=vancomycin; IniMTT=start of MTT treatment; EndMTT=End of MTT treatment; pl=placebo.

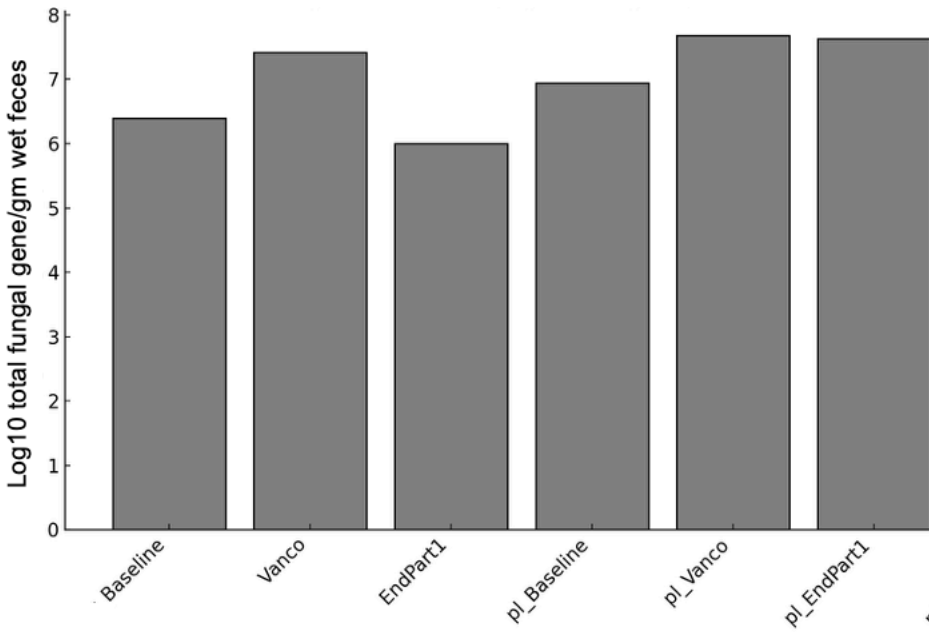

Figure S20: Quantitative measurement (qPCR) of total fungal 18S rRNA gene (log10) copy/gm wet feces in PTHS children before and after MTT. Blue color represents TD, and red color for ASD participants. MTT=Microbiota Transplant Therapy; Vanco=vancomycin; EndPart1=End of MTT treatment in Part-1; pl=placebo. Only two participants from each group showed detectable amplification. No amplification was observed at other timepoints.

Microbiome Culture Results: Culture tests were semi-quantitative tests scored on a range of 0 to 4+. For *Clostridium* the definitions of those scores are:

0: no species detectable
1+ 10^1 to 10^2 /gram of contents
2+ 10^2 to 10^4 /gram of contents
3+ 10^5 to 10^6 /gram of contents
4+ $>10^6$/gram of contents
So, there is approximately 10-100x difference between each level.

We also submitted samples from 17 healthy TD children (verified by medical history and SRS scores in the normal range) without GI symptoms. We used those children to establish a tentative reference range for potential harmful clostridia as: 1+ *C. difficile*, 1+ *C. bolteae*, 3+ *C. perfringens*, 3+ *Hungatella hathewayi/effluvia* (previously known at *Clostridium histolyticum*) and 1 + yeast (any species).

We found that 6 of 7 children with PTHS had elevated levels of potential harmful clostridia or yeast. 4 of 7 had elevated clostridia, 4 of 7 had yeast. Two patients had both elevated clostridia and yeast, and one had neither.

Table S35: Patients with Elevated Clostridia and Yeast

Patient	**Elevated Clostridia**	**Yeast**
PH-006	3+ *C. perfringens*	2+ *Candida sojae*
PH-009		
PH-003		1+ *Candida albicans*
PH-002	1+ *C. difficile*	
PH-004	3+ *C. bolteae*	1+ *Candida parapsilosis*
PH-005	4+ *Hungatella hathewayi/effluvi*	
PH 001		1+ *Candida albicans*

In comparison: for 17 typically-developing (TD) children:

None had detectable yeast

1 of 17 had elevated *Clostridium* (3+ *C. perfringens* on first test, but only 2+ *C. perfringens* on 2nd test).

Overall, this data found that the children with PTHS in this study had a higher rate of dysbiosis (86%) compared to the typically developing children (6%), defined as elevated levels of potentially harmful *Clostridium* or yeast. In detail, children with PTHS had higher rates of potential harmful clostridia (57% vs 6% of controls) and yeast (57% vs 0% of controls).

Based on these results at baseline, we analyzed all of the stool samples collected during the clinical trial, as shown in the table below:

Table of levels of Clostridia and yeast throughout the study. Group A collected 6 stool samples, and Group B collected 8 stool samples. Yellow highlights are used to indicate an elevated level of clostridia or yeast; green is used to indicate no elevations of yeast or clostridia. Only 4 potential harmful clostridia are included (*C. difficile, C. bolteae, C. perfringens*, and 4+ *Hungatella hathewayi/effluvi* (formerly known as *C. histolyticum*). If *C. perfringens* or *H. hathwayi/effluvi* are present at 1+ or 2+ they are listed but highlighted in green.

Table S36: Elevated Clostridia and Yest Throughout the Study

sample dates		Day 0 (baseline)	Day 8	Day 24	Day 94 (end part 1)			Day 198 (end part 2)	Day 296 (end part 3)
sample number		st1	st2	st3	st4	no samples scheduled for group A at these		st5	st6
Group A			vancomycin days 1-10; bowel cleanse day 11; microbiota day 12-end of part 1						
Ph-006	clostridia	3+ C perfringens		1+ Clostridium perfringens					2+ Clostridium perfringens
	yeast	Candida sojae 2+		Candida albicans 1+; Candida intermedia 1+; Candida spp. 1+; Candida sojae 2+	1+ Candida sojae; 2+ Candida albicans				
Ph-009	clostridia			1+ Clostridium perfringens				1+ Clostridium perfringens	1+ Clostridium perfringens 2+ Hungatella (Clost) hathewayi/effluvi
	yeast								
Ph-003	clostridia	1+ C perfringens			1+ Hungatella (Clost) hathewayi/effluvii			1+ Clostridium perfringens	1+ Clostridium perfringens 2+ Hungatella (Clost) hathewayi/effluvii
	yeast	1C albicans	C albicans 2+						
Group B		baseline	Day 8	Day 24	Day 94 (end part 1)	Day 108 (vanco)	Day 124	Day 194 (end part 2)	Day 296 (end part 3)
sample number		st1	st2	st3	st4	st5	st6	st7	st8
ph-002	clostridia	1+ c Difficile; 2+ c perfringens	1+ Clostridium perfringens	1+ Clostridium perfringens; 1+ Hungatella (Clost) hathewayi/effluvii	1+ Clostridium perfringens			C diff 1+; 2+ clostridium perfringens	
	yeast								
Ph-004	clostrida	3+ C Bolteae	2+ Clostridium perfringens	1+ Clostridium perfringens; 2+ Hungatella (Clost) hathewayi/effluvii	1+ Hungatella (Clost) hathewayi/effluvii	1+ Clostridium perfringens	1+ Hungatella (Clost) hathewayi/effluvii	2+ Clostridium perfringens	1+ Clostridium perfringens; 1+ Hungatella (Clost) hathewayi/effluvii
	yeast	1+ C parapsilosis							
Ph-005	clostridia	4+ Hungatella (Clost) hathewaya/effluvi	2+ Hungatella (Clost) hathewayi/effluvii	2+ Clostridium bolteae; 1+ Hungatella (Clost) hathewayi/effluvi	1+ Hungatella (Clost) hathewayi/effluvii			1+ Hungatella (Clost) hathewayi/effluvii	1+ Hungatella (Clost) hathewayi/effluvi
	yeast								
Drop-Out (unable to swallow capsules consistently)									
PH 001		C perfringens 1+							
		C albicans 1+							

Interpretations:

<u>For Group A:</u>

PH-006 had elevated *C. perfringens*, and that was reduced immediately by timepoint 2 (vancomycin) and stayed absent for the rest of the study.

PH 006 also had some intestinal yeast at baseline, which increased at during the MTT (~day 24), continued at a lower level after MTT (timepoint 4), and disappeared at timepoint 5 and 6

PH 009 did not have any clostridia or yeast on the culture test at any time during the study

PH 003 had some initial yeast, which increased at timepoint 2, and then was absent at timepoints 3-5.

<u>For Group B</u>

PH-002 had a low level of *C. difficile*, which was absent at timepoints 2-6, but re-appeared at a low level at time 7, but was gone at timepoint 8.

PH-004 had a high level of *C. bolteae* at baseline, which disappeared and was not present at any later time.

PH-004 also had a low level of yeast at baseline, which disappeared and remained absent the rest of the study.

PH-005 had high levels of *Hungatella hathewayi/effluvi*, which reduced or was absent at times 2-8, but a lower level of *C. bolteae* briefly appeared at time 3

Based on Group A, it appears that MTT resulted in a reduction of clostridia, and initially increased yeast and then eliminated it.

However, Group B had some reduction of yeast and clostridia in Part 1, which is puzzling, possibly due to a temporary benefit of the bowel cleanse or limitations of the semi-quantitative culture tests. At the end of Part 2 there were no more elevated levels of clostridia or yeast.

Overall, within the limitations of semi-quantitative culture tests, the results suggest that MTT may be effective for reducing clostridia and yeast, but the bowel cleanse alone also possibly had some effect. By the end of Part 3, no children had intestinal yeast or potential harmful levels of clostridia, compared to 5 of 6 at the start of the study.

It is likely that these harmful microorganisms contributed to the GI symptoms in children with PTHS, and likely that elimination of them contributed to improvements in GI symptoms and some PTHS symptoms.

Mitochondrial Function

At baseline, the children with PTHS had much lower activity of complex IV of the electron transport chain (averaging 46% of the average of the typically-developing (TD) children, p=0.002). Five of the 6 children with PTHS had levels below the lowest TD child. A primary function of the mitochondria is to produce ATP, the primary fuel for the body and the brain. These low levels of complex IV activity suggest that impaired mitochondrial function likely contributes to the PTHS symptoms of severe intellectual disability, severe autism, severe gross & fine motor function, and severe constipation (due to impaired peristalsis). These appear to be the first measurements of mitochondrial functioning in children with PTHS; our search of PubMed revealed no hits with the search term "Pitt Hopkins Syndrome mitochondria."

Table S37: Mitochondrial Function

A

Mitochondrial Function – Complex IV	Baseline	TD	
p-value		0.002	

B

Mitochondrial Function – Complex IV	Baseline	End Part 1	End Part 2
Group A	3.45	4.33	5.13
Group B	2.95	2.93	4.35
p-value*		0.14	
Cohen's d		1.05	

At the end of Part 1, Group A had 26% improvements in their complex IV activity, vs. -1% for Group B, with a Cohen's D effect size of 1.05 (large).

At the end of Part 2, Group A had additional improvements in their complex IV activity (49% compared to baseline), and Group B also improved after starting MTT treatment (47% increase compared to baseline).
At the end of Part 2, Group A and B averaged 68% of the average of the TD group, vs 46% at baseline.
Comparing the treatment portion of the study (Group A baseline to Part 1, Group B Part 1 to Part 2), there was a 36% increase in complex IV activity, p=0.08.

Examination of each individual child reveals that 4 of the 6 children had increases in complex IV activity, one had a small decrease, and one had little change.

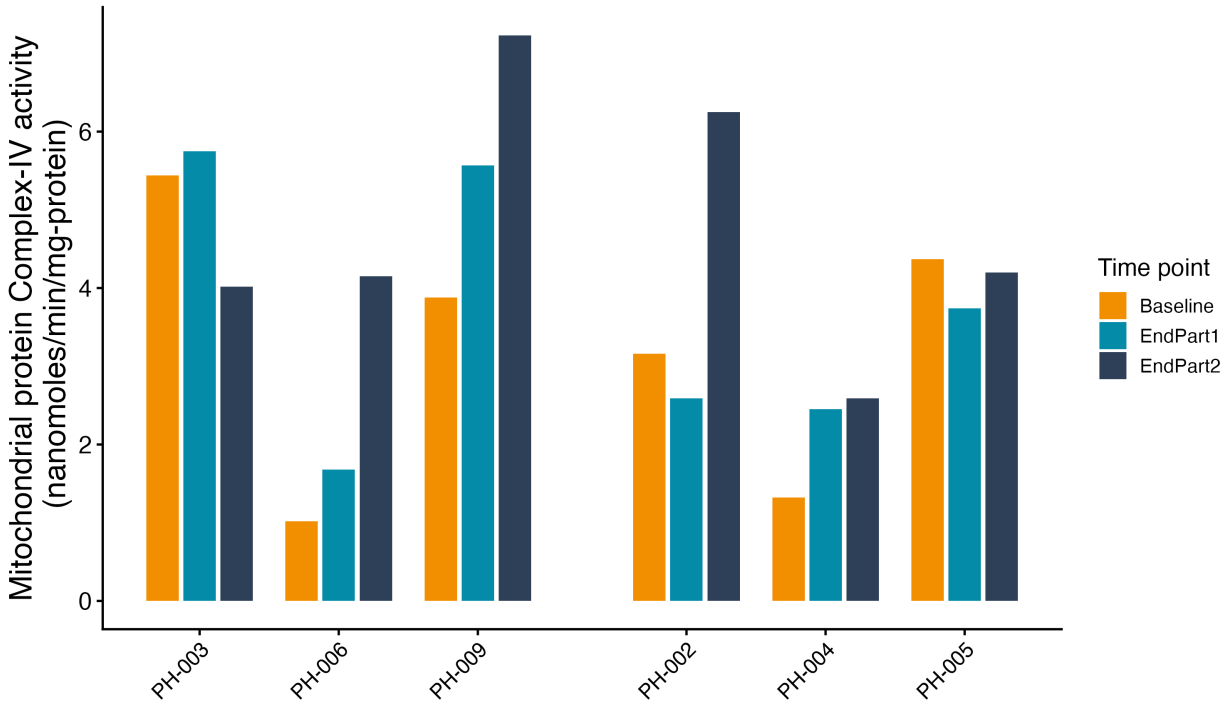

Figure S21: Complex IV activity for each study patient. Group A is on the left (first three), and Group B is on the right (last three). Group A participants; PH-003, PH-006, PH-009, and Group B participants; PH-002, PH-004, PH-005.

Overall, these results suggest that MTT substantially improved Complex IV functioning, shifting it closer to normal functioning, and this improvement may explain some of the improvements in gastrointestinal and PTHS symptoms.
Mitochondria: Mitochondrial testing revealed significantly impaired functioning of Complex IV of the electron transport chain, and MTT resulted in partial improvement from 46% to 68% of the average of typical healthy children. The impairment in mitochondrial functioning likely

contributes to many of the symptoms of PTHS, including intellectual disability, autism, gross/fine motor impairment, and constipation.
Interpretation: MTT substantially changed the microbiome, and thereby improved GI symptoms including incomplete evacuation and bloating which in turn resulted in substantial reduction of GI pain, which in turn improved many moderate to severe PTHS symptoms. This also suggests that abnormal gut bacteria contributed to these GI and PTHS symptoms. It seems likely that elimination of pathogenic bacteria/yeast and their harmful metabolites, and improvement in mitochondrial function, contributed to symptom improvement.

MTT was also effective in partially improving mitochondrial function (complex IV), which was very low in children with PTHS compared to controls, possibly due to the elimination of bacterial and yeast toxins.

MTT also resulted in partial improvement of many core and associated symptoms related to Pitt Hopkins syndrome. The improvement may be due to reduction of GI pain, reduction of bacterial/yeast toxins and neurotoxins, and improvement in mitochondrial function.

The mitochondrial measurements revealed for the first time that children with PTHS have substantial impairment of complex IV, and that MTT was able to substantially improve it (large effect size).

Overall, MTT seems to be safe, well-tolerated, and effective in reducing GI symptoms, improving many PTHS symptoms, and improving biomarkers for GI health and mitochondrial function, with benefits lasting at least 3-6 months after treatment stopped.

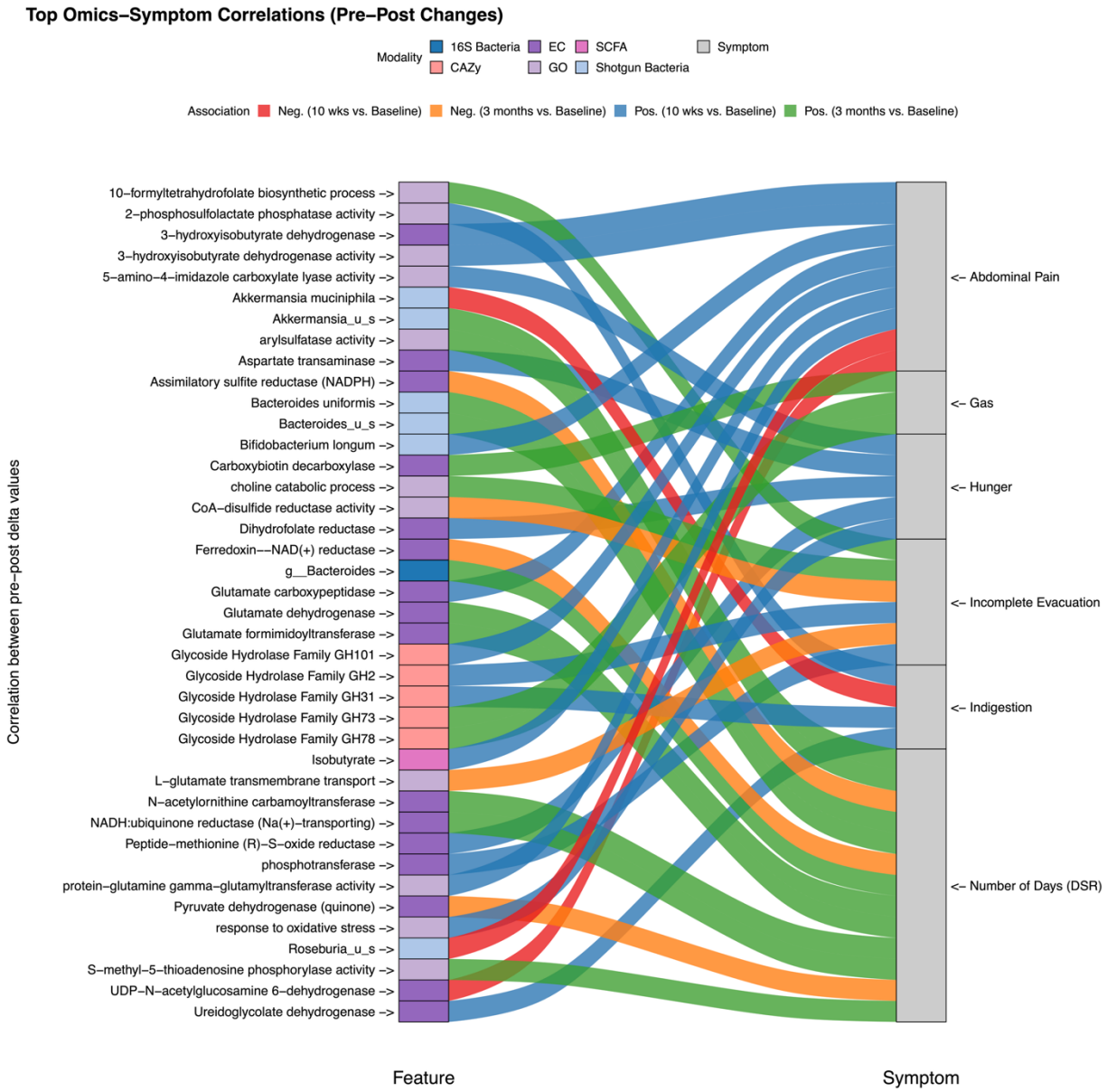

Figure S22: Correlation between Δ (delta) values of different omics features and symptoms. Modality panel shows the different features correlated with symptoms, and association panel shows type of correlation for different timepoints. Neg=negative correlation; Pos=positive correlation.