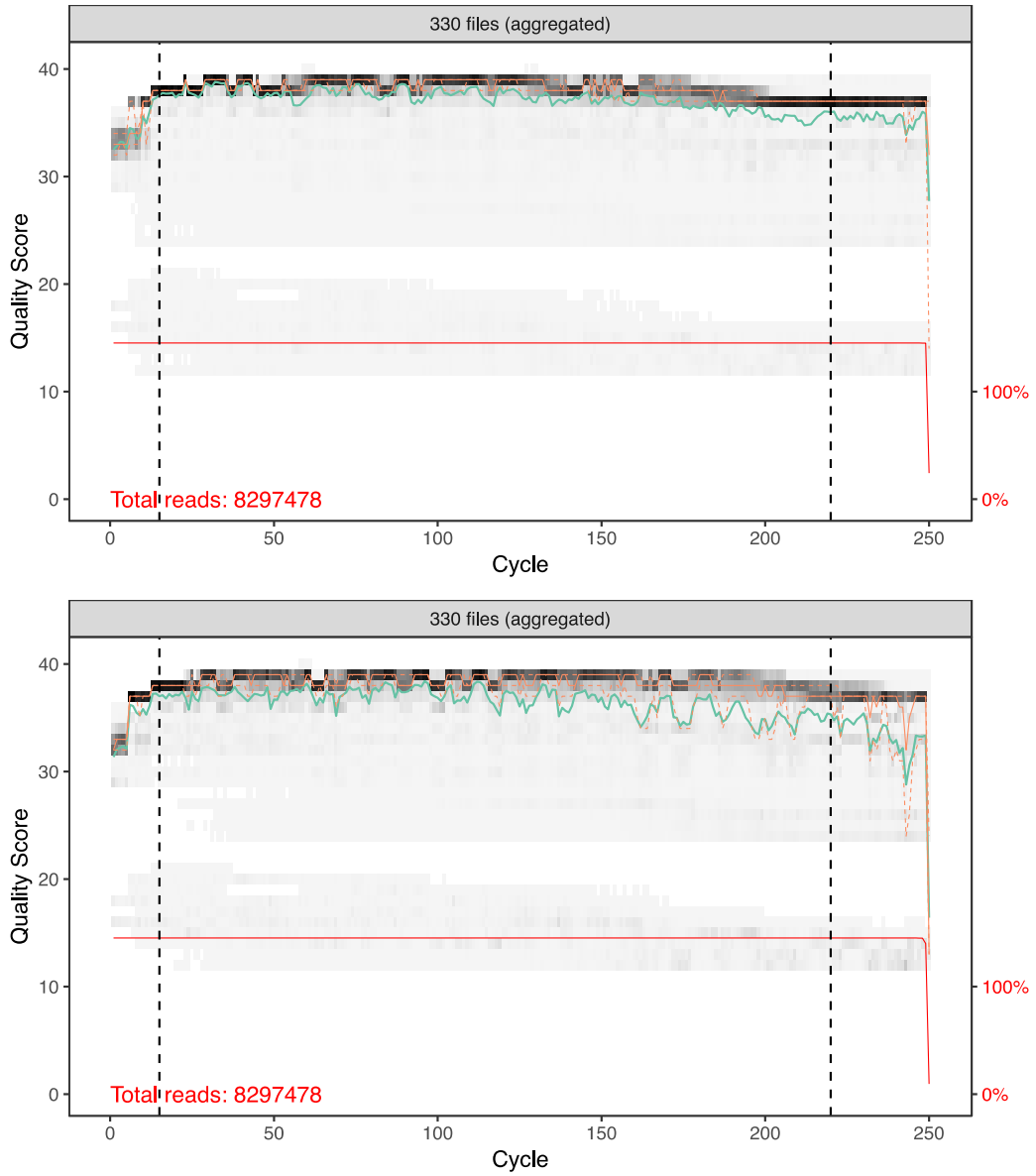
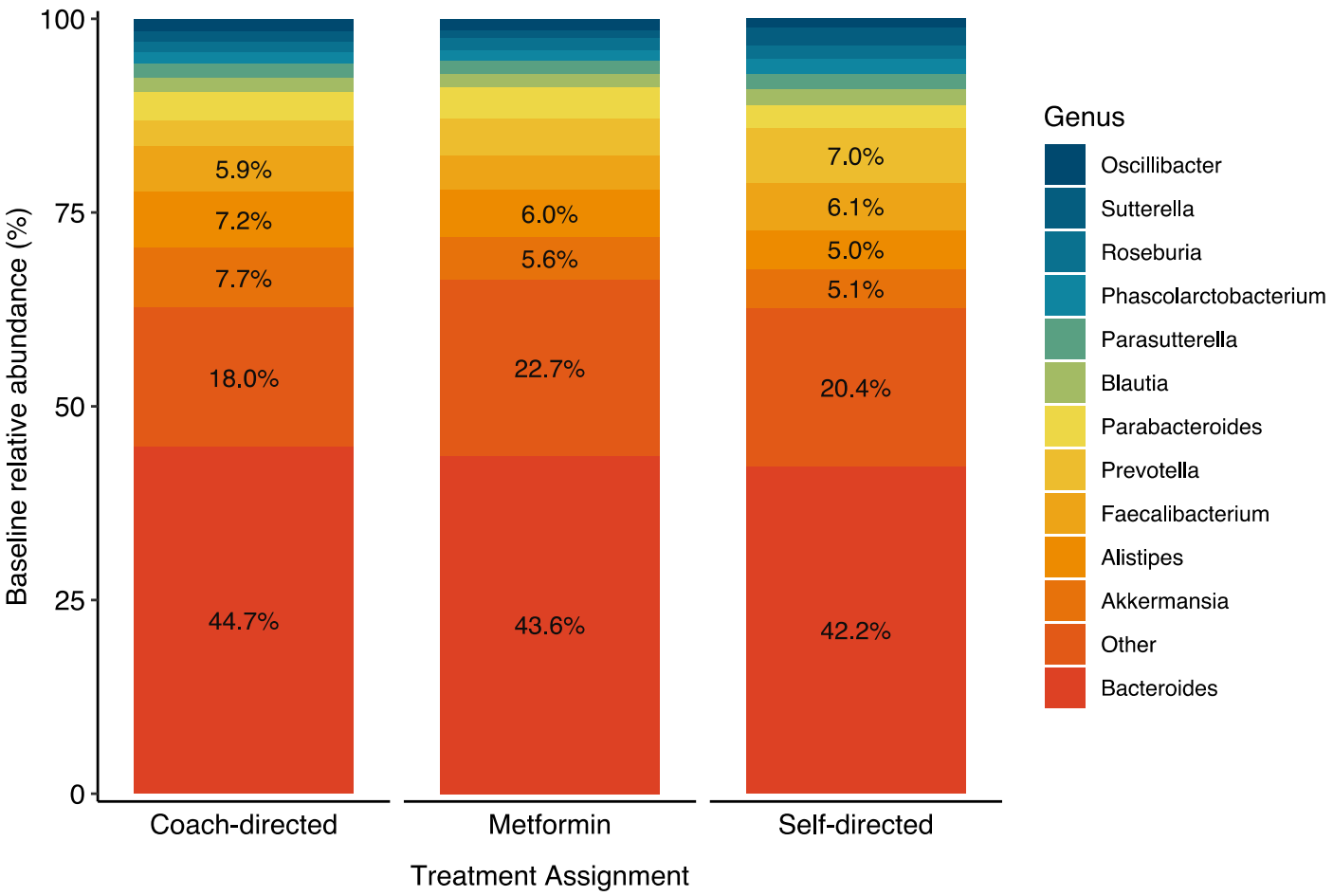
Supplemental Figures

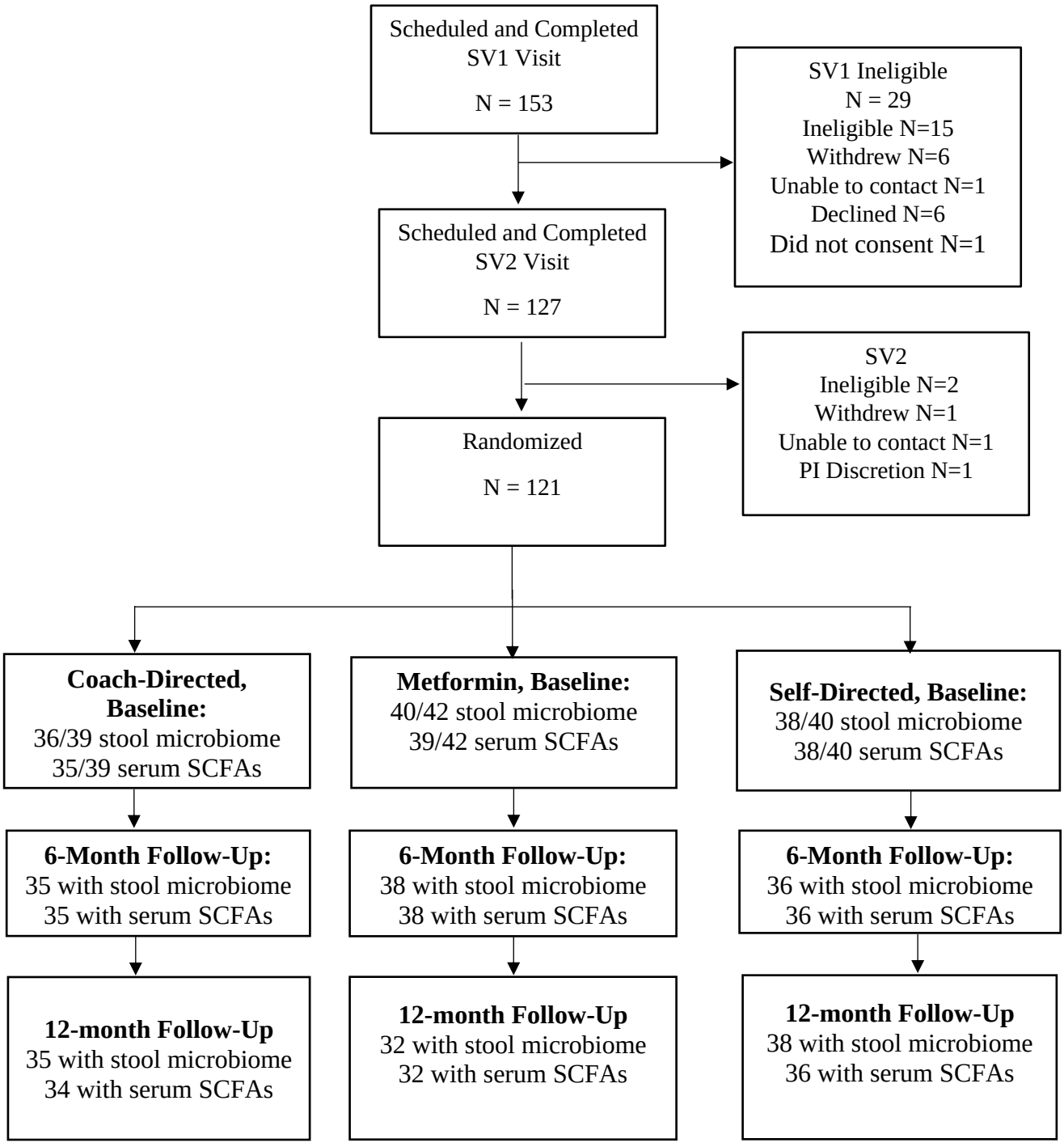
Supplemental Figure 1. Aggregate positional quality of 16S rRNA amplicon sequence reads. Forward and reverse reads are separated into top and bottom panels, respectively. The left and right trimming positions for the forward read are indicated with a vertical black line.



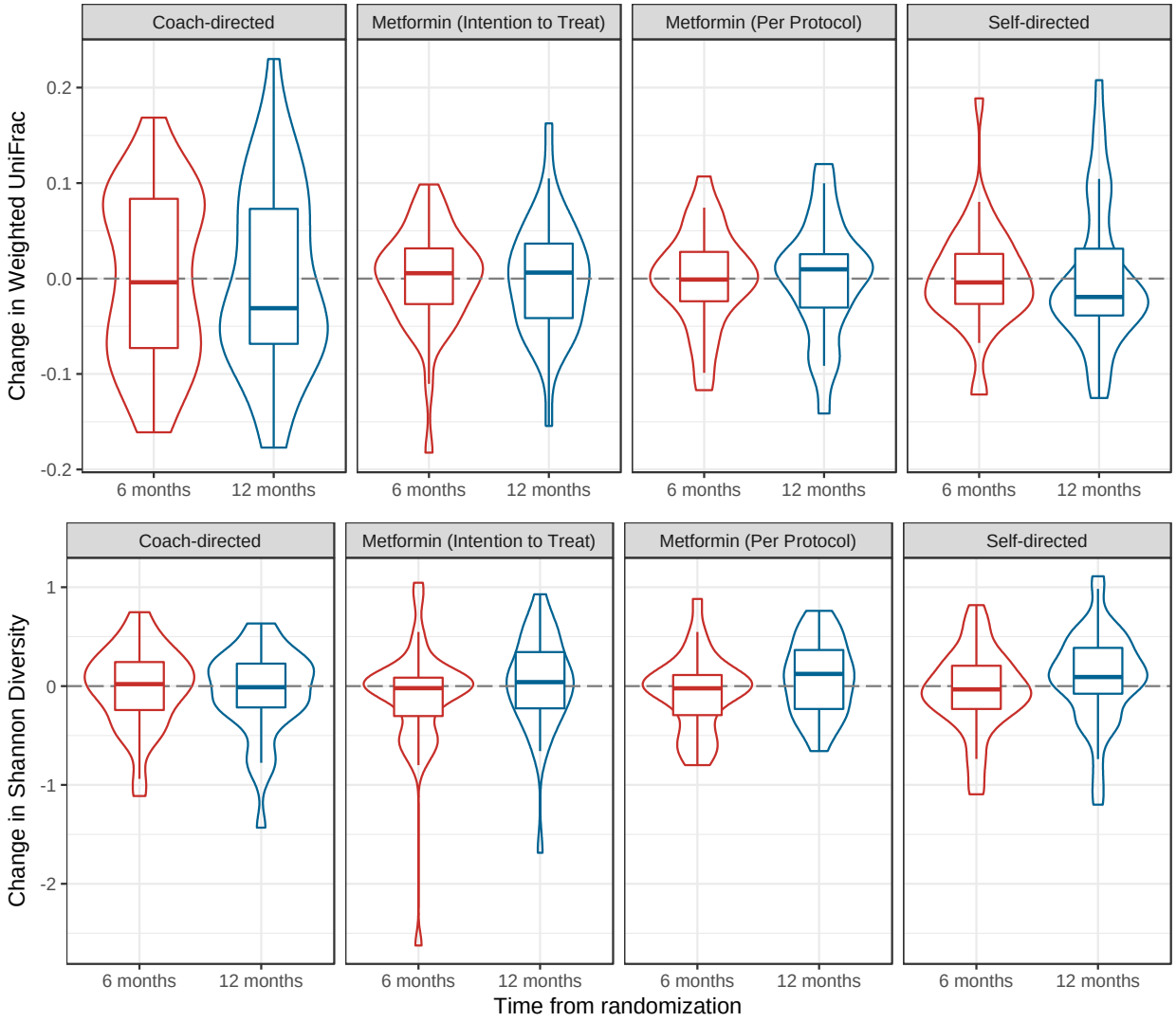
Supplemental Figure 2. Relative abundances of the top 12 bacterial genera by treatment arm at baseline. Genera with a mean relative abundance of at least 5% are labeled with % abundance.



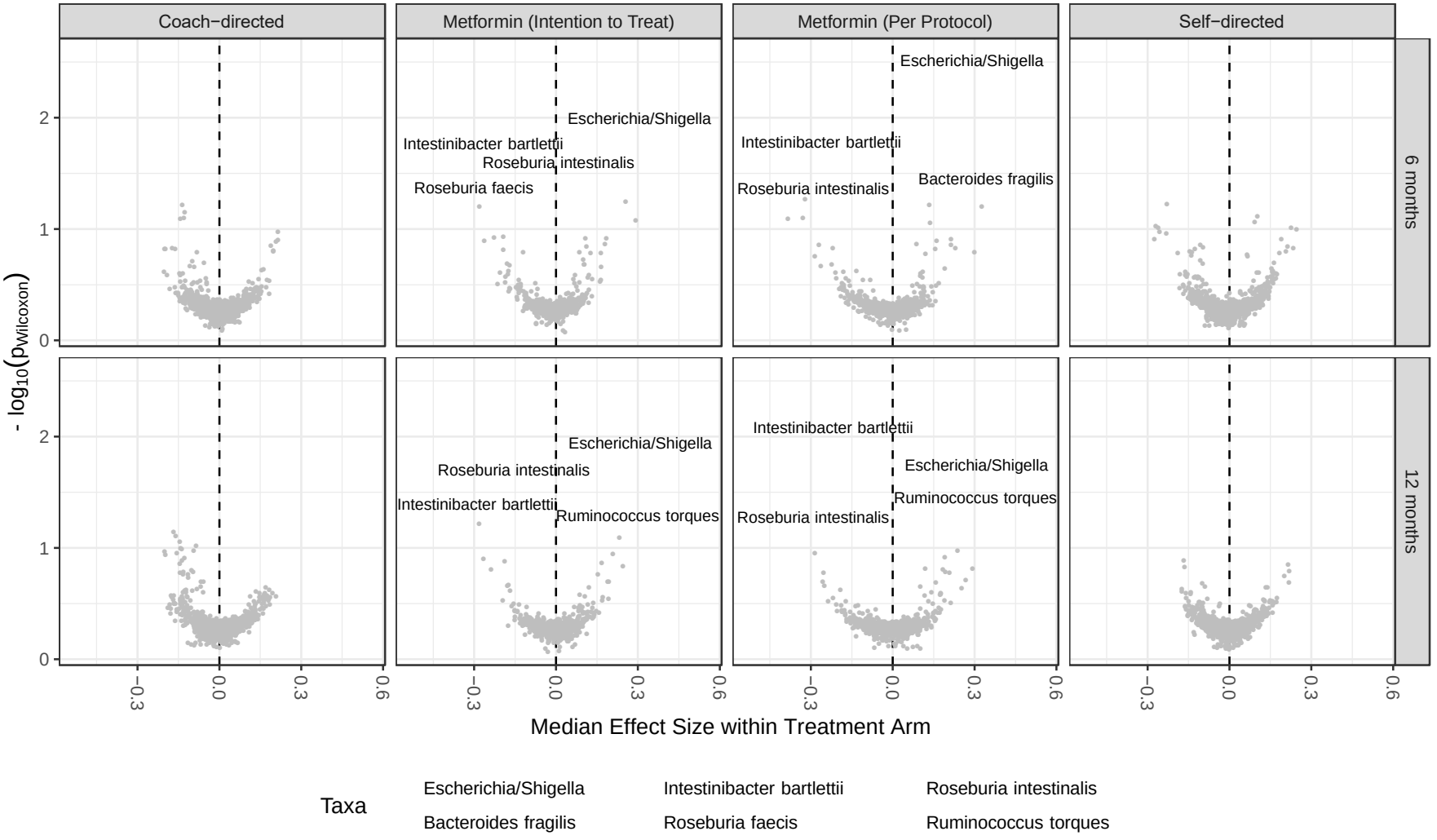
Supplemental Figure 3: SPIRIT Consort Diagram.



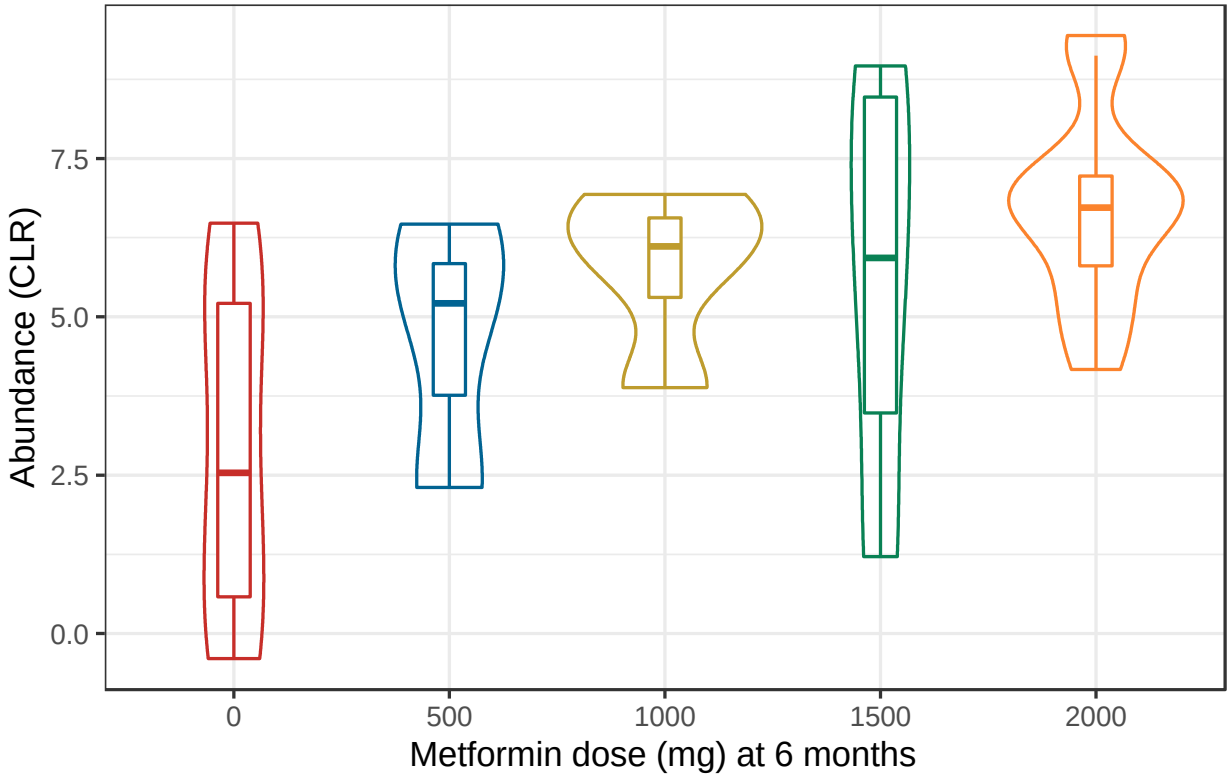
Supplemental Figure 4. Change in Weighted UniFrac distance (bottom row), a measure of microbiome beta diversity, (top row) and Shannon Diversity index, a measure of microbiome alpha diversity, between baseline and 6 months and baseline and 12 months within each treatment arm. For the metformin arm, a separate 'per protocol analysis' was also conducted to examine the effect in participants that were taking at least 500 mg/day metformin at the time of study visit.



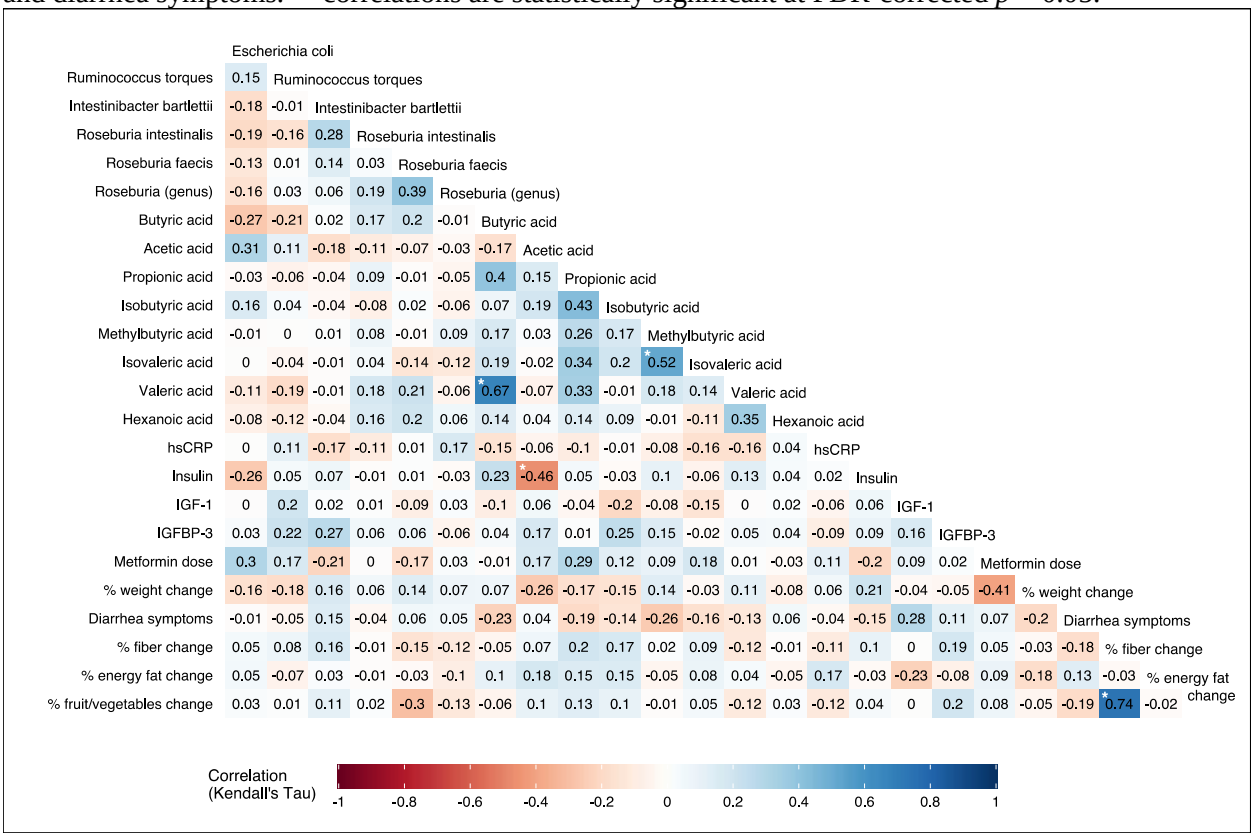
Supplemental Figure 5. Change in relative abundance of gut microbiota amplicon sequence variants between baseline and 6 months (top row) and baseline and 12 months (bottom row) in coach-directed weight loss arm, metformin arm, and self-directed weight loss arm. For the metformin arm, a separate 'per protocol analysis' was also conducted to examine the effect in participants that were taking at least 500 mg/day metformin at the time of study visit. Colored amplicon sequence variants are statistically significant at $p < 0.05$. Colored dots to the left are higher in relative abundance at baseline and colored dots to the right are higher at either 6 months or 12 months. *Sample sizes by treatment arm: Coach-directed baseline (n=36), 6 months (n=35), & 12 months (n=35); Metformin baseline (n=40), 6 months (n=38), & 12 months (n=32); Self-directed baseline (n=38), 6 months (n=36), & 12 months (n=38).*



Supplemental Figure 6. Box-and-whisker plot showing the relative abundance of the genus *Escherichia* according to the dose of metformin that participants in the metformin arm were able to tolerate at the 6 month visit.



Supplemental Figure 7. Kendall Tau correlation plot within the metformin arm (n=38), showing the correlation of change in ASVs that were significantly altered by metformin treatment at the 6-month visit with 6-month change in: serum SCFA concentrations, insulin, insulin like growth factor-1 (IGF-1), insulin like growth factor binding protein 3 (IGFBP-3), % weight, dietary factors, and metformin dose and diarrhea symptoms. *=correlations are statistically significant at FDR-corrected $p < 0.05$.



Supplemental Figure 8. Percent (%) of self-reported gastrointestinal symptoms at each study visit according to treatment arm.

Supplemental Table 1. Comparison of functional pathway richness by time in the metformin arm, using data from whole-genome shotgun metagenomic sequencing. Functional profiles were summarized into pathways using the Metacyc pathway scheme. Approximately 69.84% of reads were mapped to functional genes. Difference in pathway richness (number of unique pathways) was calculated using a generalized linear model. Data was from eight participants selected from the metformin arm. In addition to inter-individual variation, functional pathway richness was significantly increased by metformin between baseline and 6 months. P value is from chi-squared test and is not FDR corrected.

	Degrees of freedom	Deviance	Residual degrees of freedom	Residual Deviance	P value
Subject	7	30.01	8	42.34	<0.0001
Timepoint (6 month vs. baseline)	1	21.26	7	21.08	<0.00001

Supplemental Table 2. Analysis of the effect of metformin on the variation of functional groups by time, using data from whole-genome shotgun metagenomic sequencing. Permutational multivariate analysis of variance using distance matrices (PERMANOVA, using the *adonis* R function) determined the significance of differences between time points. The R^2 represents the proportion of the variability that is explained by those factors. Residuals represent the unexplained variation. In addition to inter-individual variation, functional pathway richness was significantly altered by metformin between baseline and 6 months. Participant differences explained around 55% of the variation while timepoint differences explained around 19% of the functional profile variation. P values are not FDR corrected.

	Degrees of freedom	Sum of squares	Mean squares	F model	R^2	P value
Subject	7	0.224	0.032	2.096	0.55	0.015
Time point (6 month vs. baseline)	1	0.078	0.078	5.083	0.19	0.003

Supplemental Table 3. Mean change (95% confidence interval) in CLR-transformed bacteria abundances from baseline to 6 months and baseline to 12 months between treatment arms. 95% confidence intervals that do not contain zero (0) are statistically significant at $p<0.05$. Sample sizes by treatment arm: Coach-directed baseline (n=36), 6 months (n=35), & 12 months (n=35); Metformin baseline (n=40), 6 months (n=38), & 12 months (n=32); Self-directed baseline (n=38), 6 months (n=36), & 12 months (n=38).

Bacteria	Coach-directed *vs.* Self-directed		Metformin *vs.* Self-directed		Metformin *vs.* Coach-directed	
	6 months	12 months	6 months	12 months	6 months	12 months
Escherichia coli	0.24 (-0.91, 1.39)	0.31 (-0.80, 1.43)	1.97 (0.84, 3.10)	2.48 (1.34, 3.61)	1.73 (0.57, 2.89)	2.16 (0.99, 3.33)
Intestinibacter bartlettii	0.28 (-0.59, 1.14)	0.50 (-0.42, 1.41)	-1.21 (-2.04, -0.38)	-0.87 (-1.78, 0.03)	-1.49 (-2.36, -0.62)	-1.37 (-2.32, -0.41)
Roseburia (genus)	0.54 (-0.25, 1.33)	-0.01 (-0.73, 0.72)	-0.10 (-0.87, 0.67)	-0.80 (-1.54, -0.06)	-0.64 (-1.43, 0.15)	-0.80 (-1.56, -0.03)
Roseburia faecis	0.09 (-0.86, 1.04)	-0.69 (-1.70, 0.32)	-0.70 (-1.63, 0.23)	-1.07 (-2.10, -0.04)	-0.79 (-1.74, 0.16)	-0.38 (-1.44, 0.68)
Roseburia intestinalis	0.56 (-0.44, 1.57)	0.26 (-0.71, 1.24)	-0.85 (-1.82, 0.13)	-1.30 (-2.29, -0.31)	-1.41 (-2.41, -0.41)	-1.56 (-2.58, -0.53)
Ruminococcus torques	0.50 (-0.46, 1.47)	0.50 (-0.43, 1.42)	0.97 (0.03, 1.91)	1.23 (0.29, 2.17)	0.47 (-0.49, 1.43)	0.73 (-0.24, 1.69)

Supplemental Table 4. Differential abundance testing of taxonomic groups from the whole-genome shotgun sequencing of stool microbiome, comparing mean relative abundance (%) at baseline and six month visits among eight participants randomized to metformin and selected for whole-genome shotgun sequencing. P values were from Wilcoxon ranked sum test and not FDR corrected.

Species	Mean (%) at Baseline	Mean (%) at 6 Months	log 2 Fold Change	P value
Bifidobacterium longum	0.58	0.06	-3.05	0.036
Bacteroides uniformis	18.56	9.43	-0.98	0.008
Eubacterium rectale	3.05	0.28	-3.39	0.008
Roseburia intestinalis	0.18	0	-4.02	0.036
Faecalibacterium prausnitzii	2.88	0.65	-2.14	0.008
Escherichia coli	1.04	5.58	2.41	0.008

Differential abundance was tested using Wilcoxon signed ranks test. These tests evaluated the distribution of bacterial species between timepoints (baseline and six months). Six (out of 156) of the detected species were differentially abundant between timepoints.

Supplemental Table 5. Differential abundance testing of functional groups, among the eight participants in the metformin arm selected for shotgun metagenomic analysis we agnostically conducted differential abundance testing of functional pathways. We identified 62 pathways that were significantly different between baseline and the 6-month timepoint, according to a Wilcoxon signed rank test after FDR correction (Padj). For detailed description of the pathways go to the BioCyc website (https://biocyc.org/).

KEGG Pathways	Baseline Mean	6 Month Mean	log 2 Fold Change	P	Padj
AST-PWY: L-arginine degradation II (AST pathway)	1.65	19.90	2.98	<0.01	<0.05
UBISYN-PWY: superpathway of ubiquinol-8 biosynthesis (prokaryotic)	2.00	22.48	2.97	<0.01	<0.05
PWY0-1533: methylphosphonate degradation I	2.00	16.53	2.55	<0.01	<0.05
PWY-6708: ubiquinol-8 biosynthesis (prokaryotic)	3.55	24.40	2.48	<0.01	<0.05
PWY-5855: ubiquinol-7 biosynthesis (prokaryotic)	3.55	24.40	2.48	<0.01	<0.05
PWY-5856: ubiquinol-9 biosynthesis (prokaryotic)	3.55	24.40	2.48	<0.01	<0.05
PWY-5857: ubiquinol-10 biosynthesis (prokaryotic)	3.55	24.40	2.48	<0.01	<0.05
PWY-7409: phospholipid remodeling (phosphatidylethanolamine, yeast)	2.86	20.44	2.47	<0.01	<0.05
PWY-4041: γ-glutamyl cycle	6.68	41.17	2.46	<0.01	<0.05
PWY-5367: petroselinate biosynthesis	28.69	4.72	-2.38	<0.01	<0.05
PWY-6629: superpathway of L-tryptophan biosynthesis	7.33	42.07	2.37	<0.01	<0.05
PWY0-1241: ADP-L-glycero-β-D-manno-heptose biosynthesis	4.68	28.05	2.35	<0.01	<0.05
TCA-GLYOX-BYPASS: superpathway of glyoxylate bypass and TCA	8.16	43.63	2.29	<0.01	<0.05
*FASYN-INITIAL-PWY: superpathway of fatty acid biosynthesis initiation (**E. coli**)*	10.14	51.50	2.24	<0.01	<0.05
PWY-7315: dTDP-N-acetylthomosamine biosynthesis	7.82	40.57	2.24	<0.01	<0.05
PWY-7204: pyridoxal 5'-phosphate salvage II (plants)	14.91	73.22	2.22	<0.01	<0.05
PWY-7388: octanoyl-[acyl-carrier protein] biosynthesis (mitochondria, yeast)	9.45	47.59	2.22	<0.01	<0.05
PWY-7254: TCA cycle VII (acetate-producers)	5.69	29.96	2.21	<0.01	<0.05
P105-PWY: TCA cycle IV (2-oxoglutarate decarboxylase)	8.60	42.98	2.20	<0.01	<0.05
PWY0-862: (5Z)-dodec-5-enoate biosynthesis	12.00	58.16	2.19	<0.01	<0.05
ECASYN-PWY: enterobacterial common antigen biosynthesis	3.06	16.95	2.15	<0.01	<0.05
GLYCOL-GLYOXDEG-PWY: superpathway of glycol metabolism and degradation	3.96	20.98	2.15	<0.01	<0.05
PWY-7664: oleate biosynthesis IV (anaerobic)	13.85	64.80	2.15	<0.01	<0.05
REDCITCYC: TCA cycle VIII (helicobacter)	5.75	28.50	2.13	<0.01	<0.05
FASYN-ELONG-PWY: fatty acid elongation – saturated	15.66	71.63	2.12	<0.01	<0.05
PWYG-321: mycolate biosynthesis	14.24	64.73	2.11	<0.01	<0.05
GLYCOLYSIS-TCA-GLYOX-BYPASS: superpathway of glycolysis, pyruvate dehydrogenase, TCA, and glyoxylate bypass	14.03	61.26	2.05	<0.01	<0.05
GLYOXYLATE-BYPASS: glyoxylate cycle	8.30	37.34	2.04	<0.01	<0.05
PWY-5384: sucrose degradation IV (sucrose phosphorylase)	4.69	21.72	2.00	<0.01	<0.05
PWY-6519: 8-amino-7-oxononanoate biosynthesis I	14.61	60.78	1.99	<0.01	<0.05
KDO-NAGLIPASYN-PWY: superpathway of (Kdo)2-lipid A biosynthesis	3.32	15.98	1.98	<0.01	<0.05

PWY-6282: palmitoleate biosynthesis I (from (5Z)-dodec-5-enoate)	14.03	57.84	1.97	<0.01	<0.05
SO4ASSIM-PWY: sulfate reduction I (assimilatory)	38.10	149.11	1.94	<0.01	<0.05
ENTBACSYN-PWY: enterobactin biosynthesis	9.85	39.70	1.91	<0.01	<0.05
PWY-5723: Rubisco shunt	13.90	52.06	1.83	<0.01	<0.05
BIOTIN-BIOSYNTHESIS-PWY: biotin biosynthesis I	18.04	66.78	1.83	<0.01	<0.05
PWY-7196: superpathway of pyrimidine ribonucleosides salvage	59.96	16.29	-1.82	<0.01	<0.05
PWY0-1415: superpathway of heme biosynthesis from uroporphyrinogen-III	8.72	32.12	1.77	<0.01	<0.05
PWY-5791: 1,4-dihydroxy-2-naphthoate biosynthesis II (plants)	12.54	44.63	1.75	<0.01	<0.05
PWY-5837: 1,4-dihydroxy-2-naphthoate biosynthesis I	12.54	44.63	1.75	<0.01	<0.05
GLUCOSE1PMETAB-PWY: glucose and glucose-1-phosphate degradation	14.80	49.63	1.68	<0.01	<0.05
PWY-5838: superpathway of menaquinol-8 biosynthesis I	23.79	78.69	1.69	<0.01	<0.05
PWY-5845: superpathway of menaquinol-9 biosynthesis	21.91	70.08	1.63	<0.01	<0.05
PWY-5850: superpathway of menaquinol-6 biosynthesis I	21.91	70.08	1.63	<0.01	<0.05
PWY-5896: superpathway of menaquinol-10 biosynthesis	21.91	70.08	1.63	<0.01	<0.05
PWY-5897: superpathway of menaquinol-11 biosynthesis	33.67	100.46	1.55	<0.01	<0.05
PWY-5898: superpathway of menaquinol-12 biosynthesis	33.67	100.46	1.55	<0.01	<0.05
PWY-5899: superpathway of menaquinol-13 biosynthesis	33.67	100.46	1.55	<0.01	<0.05
PWY-5840: superpathway of menaquinol-7 biosynthesis	24.10	70.08	1.50	<0.01	<0.05
PWY-5863: superpathway of phylloquinol biosynthesis	9.70	29.50	1.51	<0.01	<0.05
PWY-5189: tetrapyrrole biosynthesis II (from glycine)	9.61	27.24	1.41	<0.01	<0.05
P441-PWY: superpathway of N-acetylneuraminate degradation	25.13	66.61	1.37	<0.01	<0.05
PWY-6628: superpathway of L-phenylalanine biosynthesis	29.53	74.29	1.30	<0.01	<0.05
PWY-5918: superpathay of heme biosynthesis from glutamate	15.37	36.23	1.19	<0.01	<0.05
PWY-6737: starch degradation V	332.36	159.82	-1.05	<0.01	<0.05
PWY-5100: pyruvate fermentation to acetate and lactate II	69.65	33.35	-1.04	<0.01	<0.05
HEMESYN2-PWY: heme biosynthesis II (anaerobic)	19.56	38.75	0.95	<0.01	<0.05
PWY-5345: superpathway of L-methionine biosynthesis (by sulfhydrylation)	42.46	81.83	0.93	<0.01	<0.05
SER-GLYSYN-PWY: superpathway of L-serine and glycine biosynthesis I	94.45	54.75	-0.78	<0.01	<0.05
PWY-1269: CMP-3-deoxy-D-manno-octulosonate biosynthesis I	132.66	218.80	0.72	<0.01	<0.05
TRNA-CHARGING-PWY: tRNA charging	198.39	123.78	-0.68	<0.01	<0.05
PWY-6163: chorismate biosynthesis from 3-dehydroquinate	304.82	258.28	-0.24	<0.01	<0.05

Supplemental Table 6. Mean change (95% confidence interval) in ln-transformed fasting serum short chain fatty acids from baseline to 6 months and 12 months between treatment arms. This table corresponds to statistics in **Figure 3**. *n* = 118 at 6 months; *n* = 113 at 12 months. 95% confidence intervals that do not contain 0 are statistically significant.

	Coach-directed *vs.* Self-directed		Metformin *vs.* Self-directed		Metformin *vs.* Coach-directed	
Short chain fatty acids	6 months	12 months	6 months	12 months	6 months	12 months
ln(Acetic Acid)	0.36 (0.11, 0.61)	0.03 (-0.25, 0.31)	0.24 (-0.004, 0.48)	0.14 (-0.14, 0.42)	-0.12 (-0.37, 0.13)	0.11 (-0.18, 0.40)
ln(Propionic Acid)	0.05 (-0.15, 0.25)	0.07 (-0.12, 0.25)	0.13 (-0.07, 0.33)	0.04 (-0.15, 0.22)	0.08 (-0.12, 0.28)	-0.03 (-0.22, 0.16)
ln(Isobutyric Acid)	0.04 (-0.13, 0.21)	0.08 (-0.05, 0.22)	0.04 (-0.13, 0.21)	0.02 (-0.11, 0.16)	-0.0001 (-0.17, 0.17)	-0.06 (-0.20, 0.08)
ln(Butyric Acid)	0.13 (-0.03, 0.29)	0.08 (-0.10, 0.26)	0.40 (0.24, 0.56)	0.12 (-0.06, 0.29)	0.27 (0.10, 0.43)	0.04 (-0.14, 0.22)
ln(Methylbutyric Acid)	-0.07 (-0.24, 0.11)	-0.24 (-0.45, -0.02)	0.05 (-0.12, 0.22)	-0.10 (-0.31, 0.12)	0.12 (-0.06, 0.29)	0.14 (-0.08, 0.36)
ln(Isovaleric Acid)	-0.02 (-0.23, 0.20)	-0.06 (-0.29, 0.17)	0.12 (-0.10, 0.33)	0.11 (-0.13, 0.34)	0.14 (-0.08, 0.35)	0.17 (-0.07, 0.40)
ln(Valeric Acid)	0.08 (-0.10, 0.26)	-0.06 (-0.23, 0.12)	0.19 (0.01, 0.37)	-0.08 (-0.26, 0.10)	0.11 (-0.07, 0.29)	-0.02 (-0.20, 0.15)
ln(Hexanoic Acid)	0.03 (-0.08, 0.14)	0.04 (-0.06, 0.14)	0.05 (-0.06, 0.16)	0.03 (-0.07, 0.13)	0.02 (-0.09, 0.13)	-0.01 (-0.11, 0.09)