Supplementary Materials

Supplementary Texts

- Study Protocol - Supplementary Text S1
- Statistical Analysis Plan - Supplementary Text S2
- Consort 2010 Checklist - Supplementary Text S3
- Supplementary Methods - Supplementary Text S4

Supplementary Tables

Genus species strain-identifier (abbreviation)	WBF-0010 (AFU)	WBF-0011 (AFU)
Clostridium beijerinckii WB-STR-0005 (CBEI)	1.6×10^{10}	1.6×10^{10}
Clostridium butyricum WB-STR-0006 (CBUT)	3.3×10^{9}	3.3×10^{9}
Bifidobacterium infantis 100 (BINF)	2.0×10^{9}	2.0×10^{9}
Akkermansia muciniphila WB-STR-0001 (AMUC)	0	1.2×10^{9}
Anaerobutyricum hallii WB-STR-0008 (EHAL)	0	9.0×10^{8}

Supplementary Table S1. **Study product strain composition and viability.** Viability values are with respect to the time of product manufacture, as determined by AFU, see supplementary text S4 for details. Probiotic strain abbreviations are provided in parenthesis and refer exclusively to the 5 strains provided by study products. Placebo was composed of colloidal silicon dioxide and provided in an identical opaque acid-resistant capsule.

System Organ Class	Placebo		WBF-010		WBF-011	
	Subjects (%)	Events	Subjects (%)	Events	Subjects (%)	Events
Cardiovascular	4 (15)	4	2 (7)	3	2 (9)	5
Gastrointestinal	7 (27)	11	7 (26)	16	4 (15)	5
Metabolic	5 (19)	8	0 (0)	0	2 (9)	2
Neurological	5 (19)	7	3 (11)	3	3 (13)	5
Ear, Nose, Throat	3 (12)	4	4 (15)	4	1 (4)	1
Dermatology	1 (4)	2	2 (7)	4	0 (0)	0
Musculoskeletal	3 (12)	3	3 (11)	4	0 (0)	0
Hematologic	1 (4)	1	0 (0)	0	1 (4)	1
Genitourinary	1 (4)	2	2 (7)	3	1 (4)	1
Infection, Viral	1 (4)	1	0 (0)	0	1 (4)	1

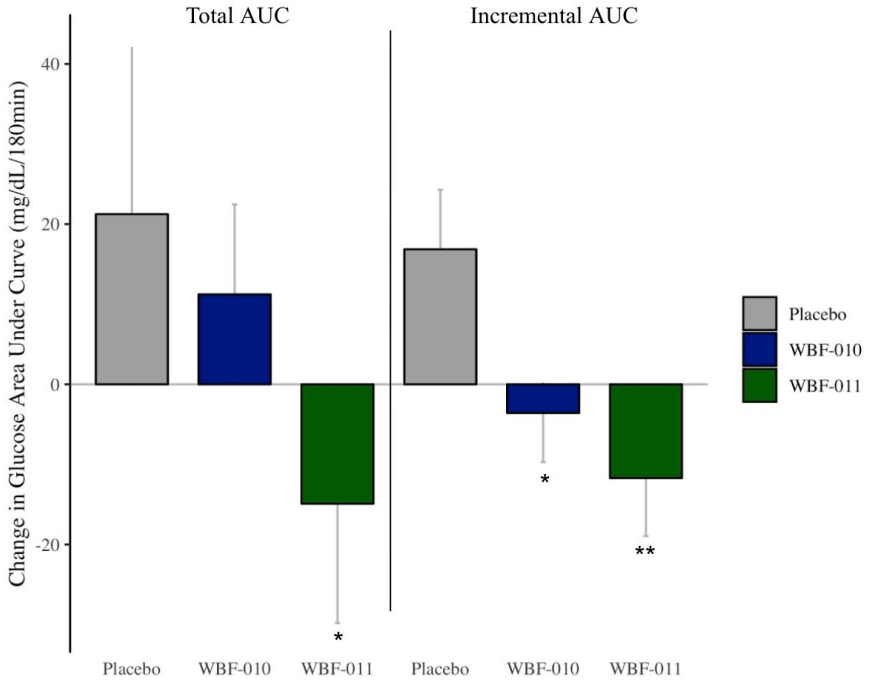
Supplementary Table S2. Adverse Events (≥4% of Subjects) by System Organ Class. Adverse events that occurred up to and including week 12 among subjects in the intent-to-treat population are included and presented by their system organ class term from the Medical Dictionary for Regulatory Activities version 18.1. Events are included if they had an onset date on or after the first day the study product was administered.

Primer pair name	Forward primer sequence	Reverse primer sequence
AMUC_AT:	GTGTTGAAGGTCACGTTGGC	GAATGGCCGACAATCGAAGC
BINF_AY:	GTCTTTCGCTAGCCGCATTG	TTCCCTTCGAAGCGCTTGAT
CBEI_AB:	CCTTAATCGGGGAAGCCCTTC	AGCATCTCTCTGTACCAGCCA
CBEI_AK:	ATGGCTGGCGGTGTAAAAGA	TGTCACCGTTTTTCCTGGCT
CBUT_AS:	TGGCGAAGTAGATAAGGCTGA	CCACACAAGCTTATGCAAAACC
EHAL_AH:	GGTCACGCGGCTGAAATTTT	CTGGTCGGCTTCCAATTGGA
EHAL_AN:	AAAGTTCTTCCGGCTGCGTA	CAACGTCAGGCAAACAAGCA

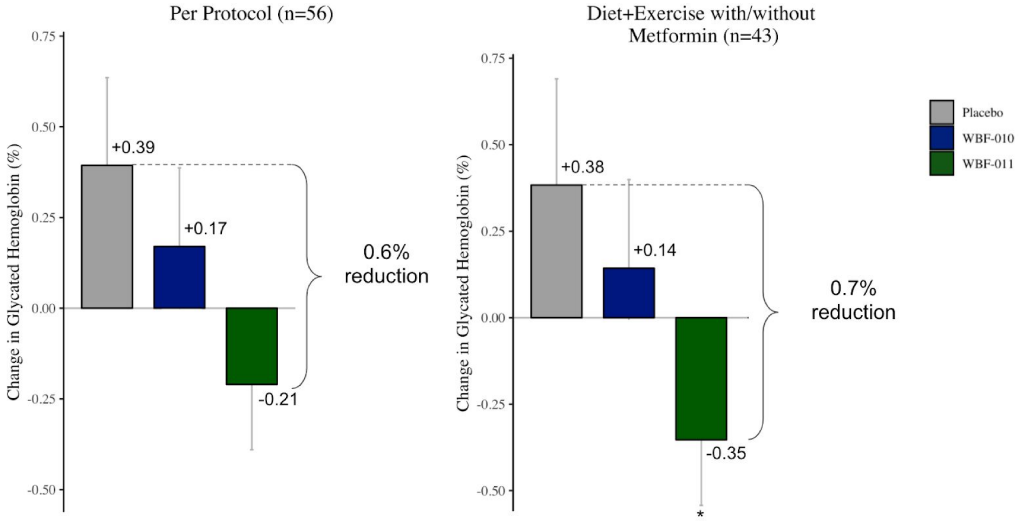
Supplementary Table S3. Primers used in qPCR. See figure 2 and supplementary text S4.

Supplementary Figures

A



B



Supplementary Figure S1. Effect of 12-week twice-daily dosing on postprandial glucose following a standard 3-hour meal-tolerance test for Per Protocol population. (A) Change in Total and Incremental Glucose Area Under Curve at 12 Weeks in Per-protocol Population (N = 58). (B) Change in Glycated Hemoglobin at 12 Weeks in Per-protocol Population (N = 56, per protocol population with two week 12 missing values) and Population Managed with Diet and Exercise With or Without Metformin (N=43). Data shown are means ± SE. *P=0.0409

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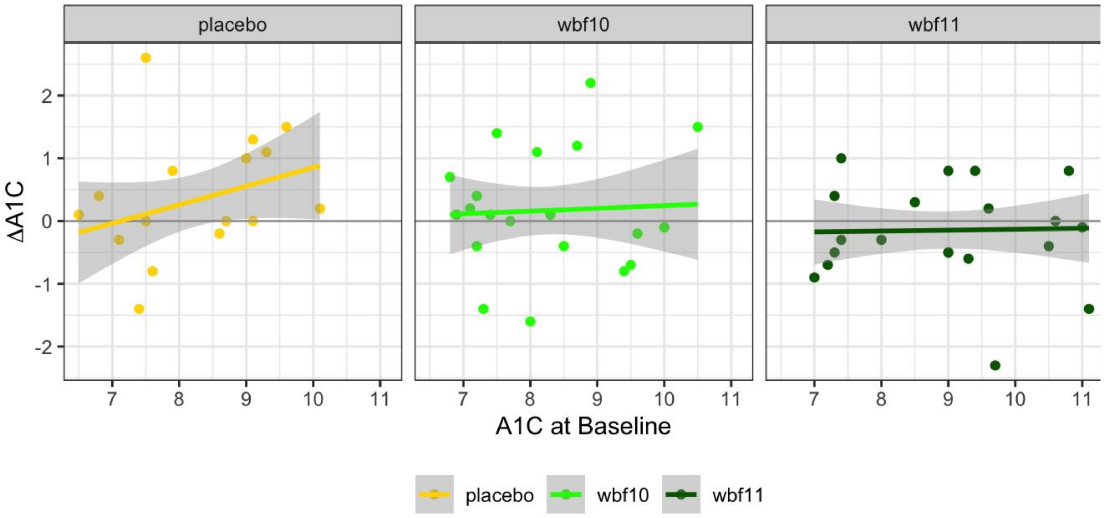

Supplementary Figure S2. Starting A1c versus 12-week change in A1c by group assignment. Placebo dropout had higher on-average starting A1c values and therefore any bias due to differential dropout would likely impact intervention effects conservatively.

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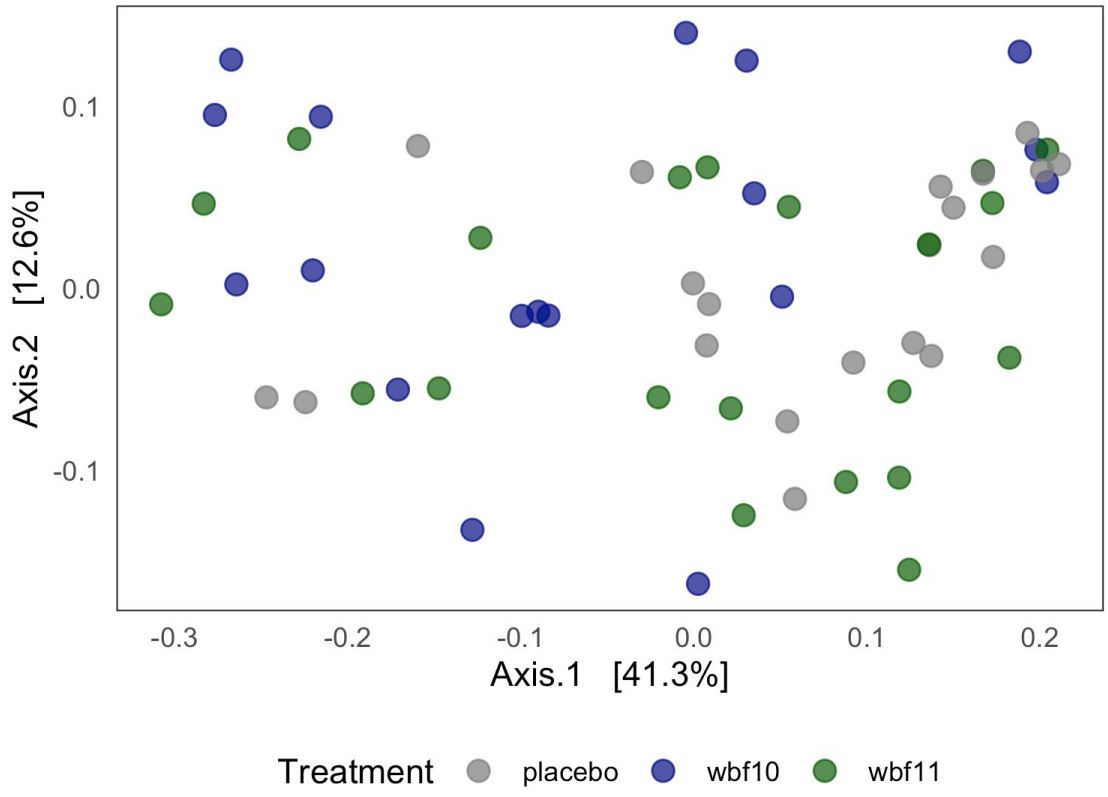
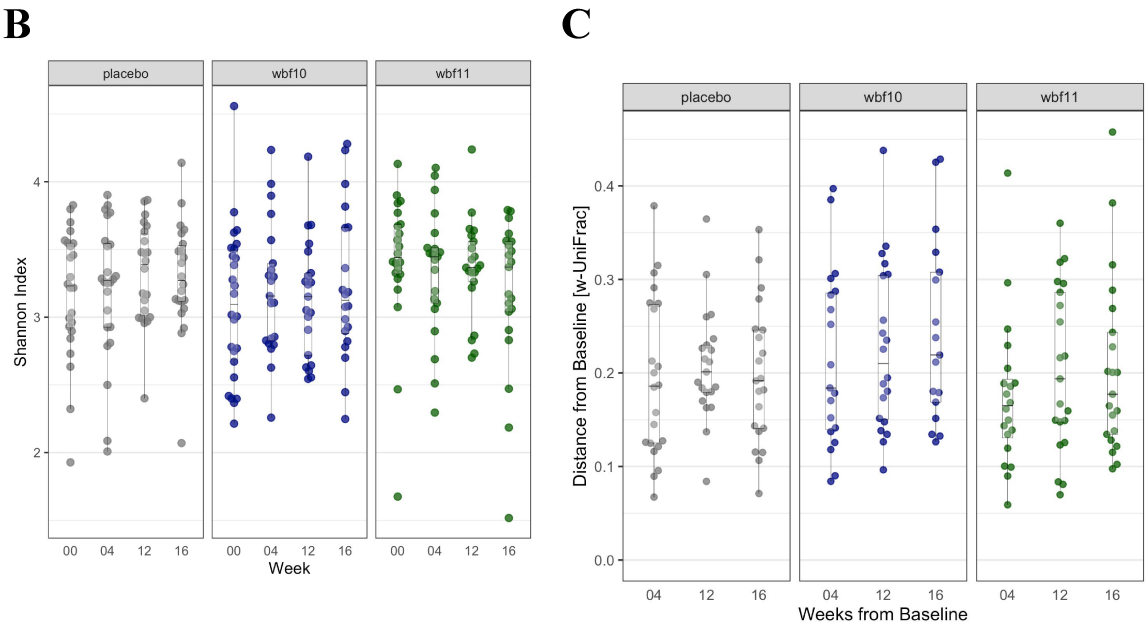
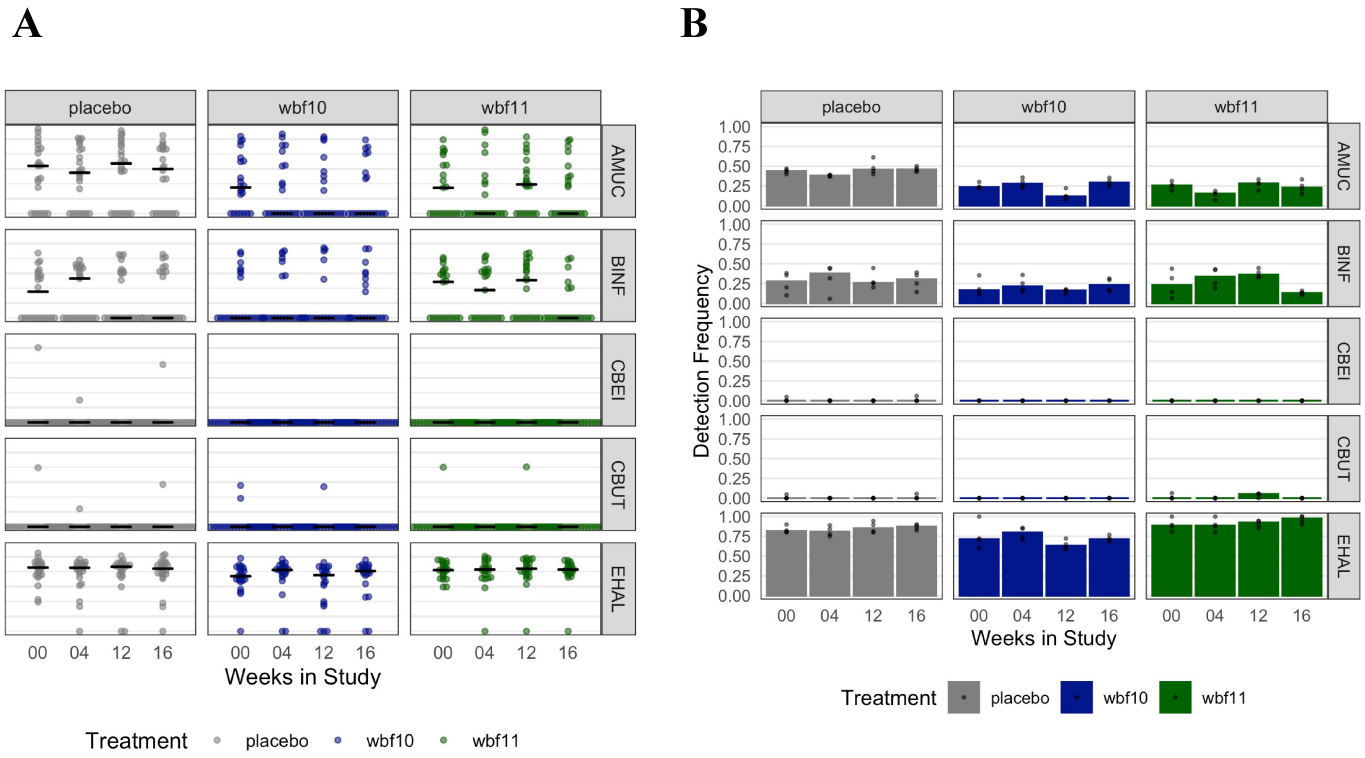
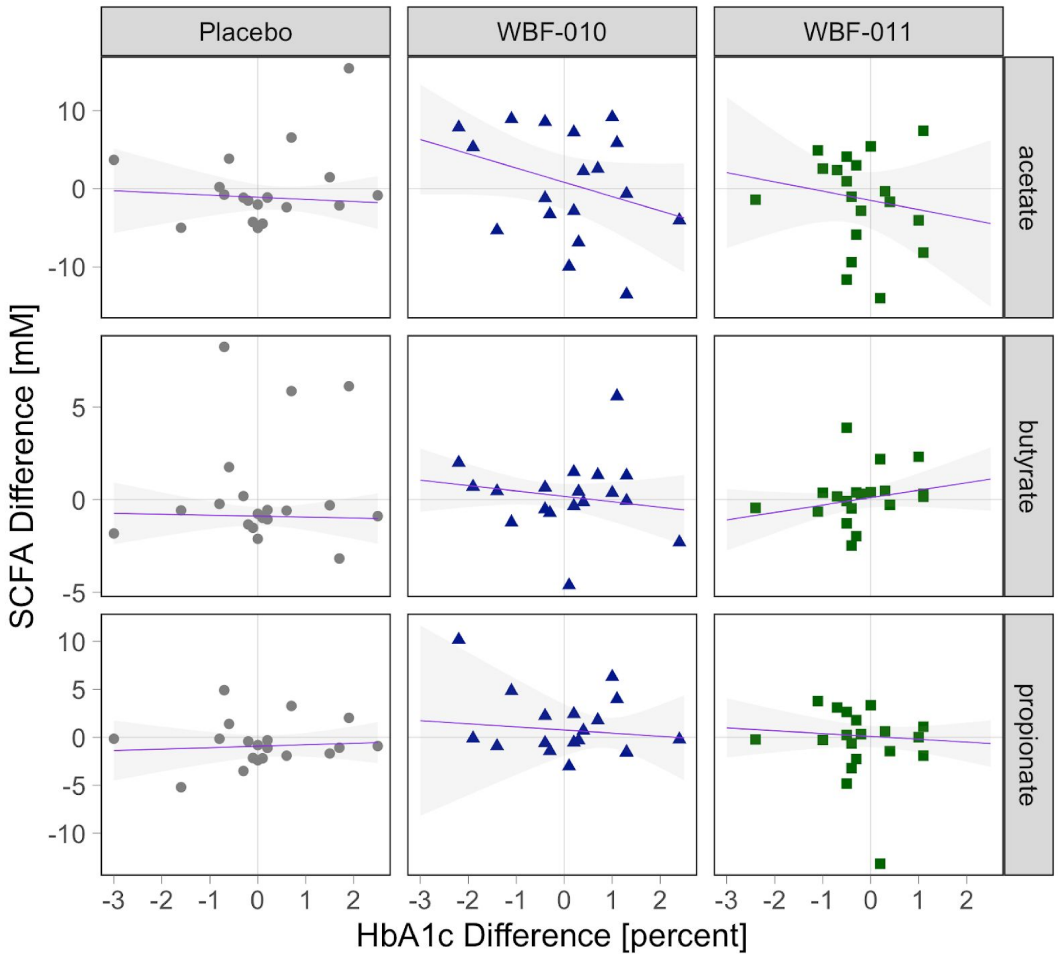

0.1
0.0
-0.1
Axis.2 [12.6%]
-0.3
-0.2
-0.1
0.0
0.1
0.2
Axis.1 [41.3%]
Treatment
placebo
wbf10
wbf11



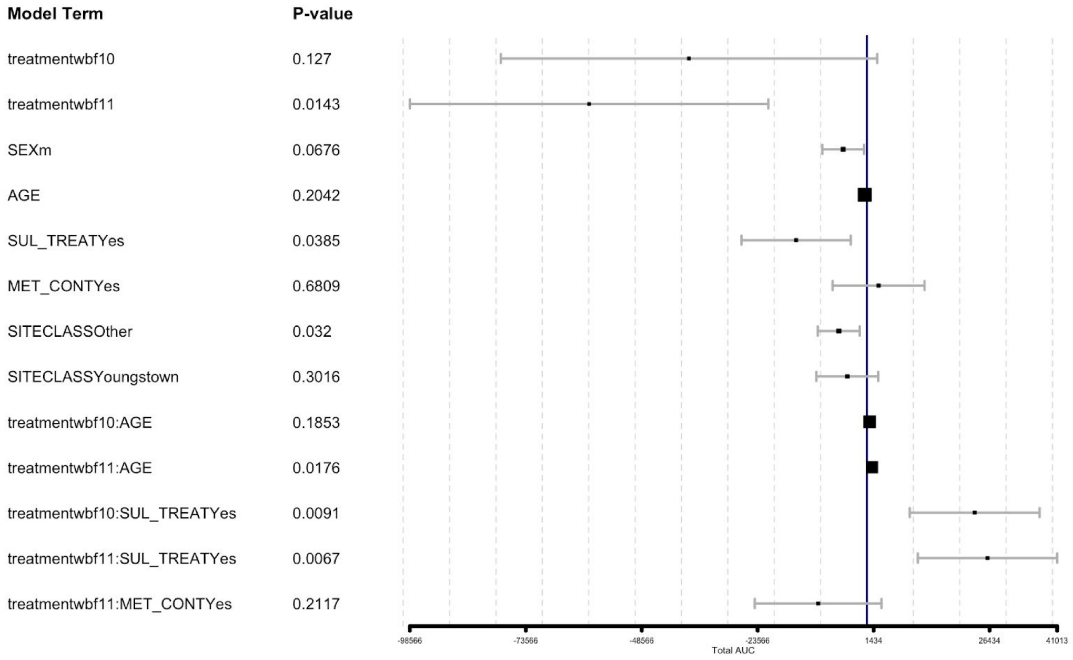
Supplementary Figure S3. Comparison of stool microbiome profiles based on 16SV4. (A) Principal Components Analysis (PCoA) plot of the weighted-UniFrac distance between baseline samples for a representative 16SV4 replicate run. (B) Alpha diversity represented as Shannon Index (median across replicate 16SV4). (C) Change in beta diversity from baseline for each subject, as median weighted-UniFrac distance across 16SV4 replicates runs. In all panels, each point represents a stool microbiome from a human subject at the indicated study collection event, and color shading indicates study group.



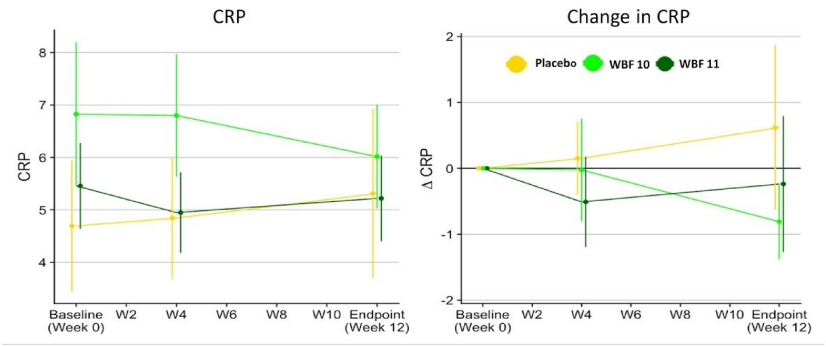
Supplementary Figure S4. 16SV4 matches pursuant to formulation strains. Sequence matches of the 16S rRNA gene V4 region of formulation strains are not unique among other known members of the species, and therefore do not necessarily disambiguate formulation strains from other closely-related endogenous strains. (A) Range of the proportions of matches between 16SV4 ASV sequences and known loci in genomes of formulation strains. Each point is the median sample proportion among 16SV4 replicate runs for a particular subject. Points held at the bottom of the axis represent a subject with no detected match for the indicated strain and time point. (B) Summarized detection frequency by study group and time. The frequency value is defined as the number of subjects with a sample proportion of the indicated strain that are greater than 0.1% divided by the total subjects in the indicated study group.



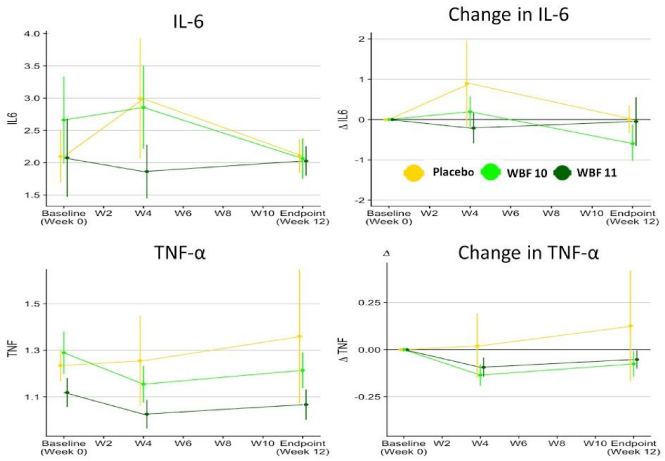
Supplementary Figure S5. Changes in SCFA and A1c. Column panels indicate trial arm, while panel rows indicate the SCFA. Thin magenta line and gray ribbon indicate the robust linear regression and associated confidence region. All SCFA difference values are in millimolar, and defined as the median value of the technical replicate differences for baseline subtracted from week-12 of the same subject. Horizontal axis indicates the change in A1c, also as baseline subtracted from week-12.



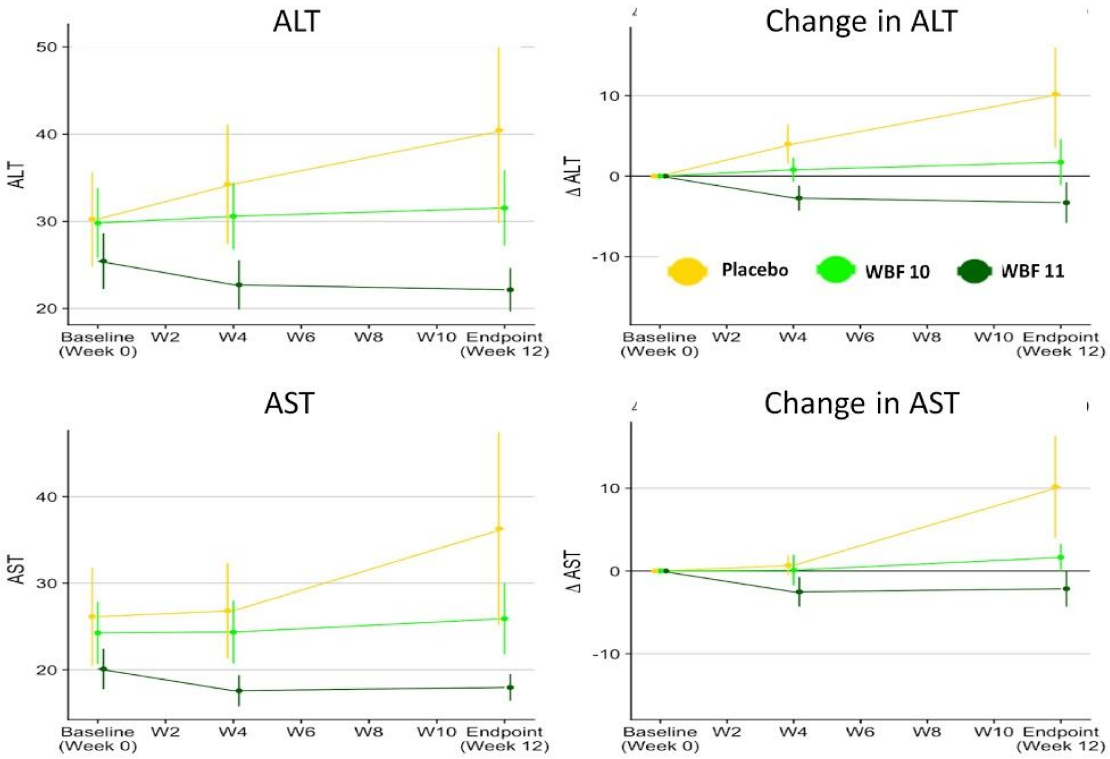
Supplementary Figure S6. Parameter estimates, p-values and 95% confidence intervals for model obtained through stepwise selection procedure. See Statistical Analysis Plan (supplementary text S2) for additional details.



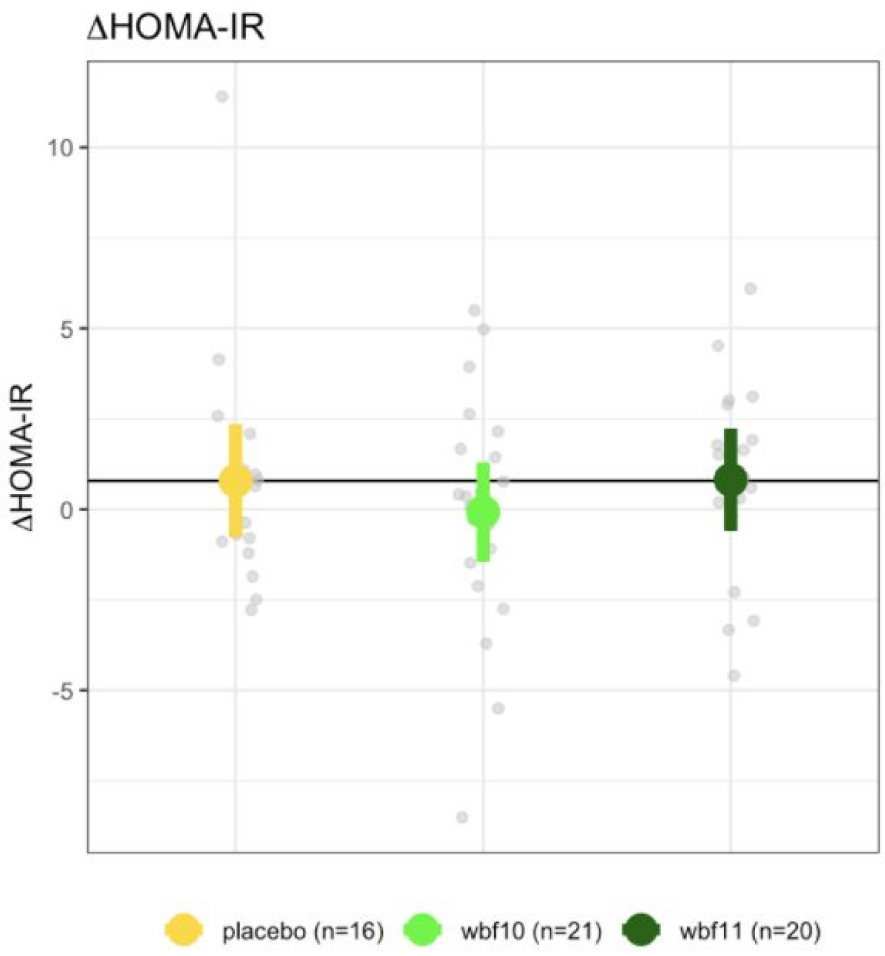
Supplementary Figure S7. C reactive protein (CRP). Mean ± SE c reactive protein concentrations over time (left panel) and change from baseline values (right panel).



Supplementary Figure S8. IL-6 and TNF-α data. Mean ± SE interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) concentrations over time (left panels) and change from baseline values (right panels).



Supplementary Figure S9. Liver Function Tests (ALT and AST plots). Changes in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels over time (left panel) and change from baseline (right panel).



Supplementary Figure S10. Homeostatic Model Assessment of Insulin Resistance (HOMA-IR). 12-week change in HOMA-IR across study groups. Each grey dot is a subject. Colored dots and bars show the mean and 95% confidence intervals.