Supplementary Material

Supplementary Methods:

Animal diets and treatments: Male germ-free (GF) Swiss-Webster mice were humanized at 8 weeks of age by oral gavage of 200µl of human fecal sample (obtained from a healthy 37 year old male donor) mixed with pre-reduced PBS or conventionalized by oral gavage of 200µl of conventional mouse cecal contents mixed with pre-reduced PBS. All mice were 12 weeks old at time of experiment and protocols were in accordance with A-PLAC, the Stanford IACUC and were fed either an autoclaved standard diet (Purina LabDiet 5K67) or custom irradiated diets containing either cellulose, FOS or glucose (Bio-Serv; Supplementary Table 1). Germ free, conventional, conventionalized or humanized Swiss Webster mice were fed either an autoclaved standard diet (Purina LabDiet 5K67) or custom irradiated diets containing either cellulose, FOS or glucose (Bio-Serv). All breeder and experimental isolators were periodically assessed for germ-free status by both culture based methods (aerobic and anaerobic) as well as 16S based PCR screens using universal primers. Sterility of multiple irradiated custom diets from this vendor (Bio-Serv) has been monitored in the lab over a four year span with no samples testing positive for contamination. Consistent with this, GF mice fed a cellulose diet did not reveal any specific product by 16S amplification using universal primers, as seen in positive controls (DNA extracted using the MoBio Ultraclean kit), again consistent with our standard surveys. It should be noted that sterility of irradiated diets may vary between vendors. For transit time experiments, mice were fed standard diet supplemented with 15% PEG 3350 (PEG, Miralax™)[1] or 0.1% loperamide hydrochloride (Sigma Aldrich, St. Louis, MO) in water[2]. Amount of daily food intake was measured at three day intervals by weighing the food before and after 3 days of consumption. To determine role of serotonin 5HT3/4 antagonist SDZ205-507 (Tocris Bioscience, Bristol, UK) was injected i.p at 20mg/kg.[3] The drug was prepared using

sterile water and passed through a 0.22 micron filter prior to introducing into the germ free isolator.

Pyrosequencing/ Data analysis: Fecal DNA was isolated and amplicons generated of the 16S V3-5 region (338F, 906R). Samples were sequenced at Duke ISGP using the Roche 454 Titanium platform. OTUs (Operational Taxonomic Units) were determined at 97% sequence similarity using uclust, taxonomy was assigned using RDP classifier against the GreenGenes database, and a phylogenetic tree was built using FastTree. The OTU table was rarified at a single sequencing depth for each sample. Samples from on standard chow and on cellulose chow were rarified at 340 sequences per sample.[4, 5] Samples from mice on cellulose diet were re-sequenced with the remaining samples and rarefied at 1239 sequences per sample. Alpha diversity was determined using PD whole tree. Beta diversity was determined using unweighted UniFrac.[6] Differences in relative abundance at different taxonomic levels were determined using ANOVA with a false discovery rate of 5%.

The 626 bp amplicons (including a unique 12bp Golay barcode[7, 8]) spanning the variable region 3-5 of bacterial 16S rRNA were generated using barcoded forward primers - 5' CGT ATC GCC TCC CTC GCG CCA TCA GNN NNN NNN NNN NGC ACT CCT ACG GGA GGC AGC A 3' which contain the 454 Life Sciences primer A sequence, a unique 12-nt error-correcting Golay barcode used to tag each amplicon (designated by NNNNNNNNNNNN), the broad-range bacterial primer 338F, and a two-base linker sequence inserted between the barcode and the rRNA primer. The reverse primer used was 906R- 5'-CTATGCGCCTTGCCAGCCCGCTCAGAACCGTCAATTCCTTTGAGTTT-3' containing the Primer B sequence.

Gastrointestinal transit time: Whole gut transit time was determined using carmine red method as previously described.[9] Non-fasted mice gavaged with 300µl of 6% carmine red

solution in 0.5% methylcellulose (filter sterilized) and had free access to food and water. Mice were habituated prior to the experiment.

Colonic contractility: Mice were anesthetized with isoflurane and a pressure transducer catheter (SPR-524 Mikro-Tip catheter; Millar Instruments, Houston,TX, 3.5Fr) was introduced into the colon 4cm proximal to the anal verge. Colonic contractions were recorded in conscious mice immediately after their placement in the restraint chamber (3.3cm diameter X 9cm length, Stoelting Co, Wood Dale, IL, USA). Each catheter was connected to pressure control unit (TC-510; Millar Instruments) and signal was amplified using a bridge amplifier (FE221; Millar Instruments) acquired via Powerlab/4sp and recorded with LabChart7. For data analysis abdominal contractions and breathing artifacts were excluded by smoothing the original trace with a time constant of 2 s. The phasic component of intracolonic pressure was extracted from the original trace as previously reported[10] by removing the direct current (DC) component with a time constant of 10 s from the 2-s smoothed original trace. Colonic contractile pressure changes were quantified by measuring the area under the curve of the phasic component of the intraluminal pressure trace (pAUC) for every minute.

Metabolomics: Fecal samples were extracted using OASIS solid phase extraction cartridges and resuspended in acetonitrile. Chromatographic separation on 5µl samples (ACQUITY Ultra performance Liquid Chromatography) and mass spectrometry operated in positive electrospray mode (Exactive; ThermoFisher Scientific) were performed. Data were collected in continuous mode and analyzed with MetaboAnalyst (www.metaboanalyst.ca).

Short chain fatty acid measurement: Frozen fecal pellets (100-800mg) were acidified (37% HCL), and SCFA's extracted (500µl diethyl ether/extraction; 2 cycles). Each sample was derivatized with N-tert-butyldimethylsilyl-N-methyltrifluoracetamide (MTBSTFA; Sigma Aldrich) and quantified using a gas chromatograph (Model 7890A; Agilent technologies) coupled to a

mass spectrometer detector (Model 5975C; Agilent technologies, Palo Alto,CA). Analyses were carried out in a split mode (1:100) on an DB-5MSUI capillary column (30 m×0.25 mm, 0.25 μm film thickness, (Agilent, Santa Clara, CA) using electronic impact (70 eV) as ionisation mode and scan in m/z 50-550 mass range. The column head-pressure was 12 p.s.i. Injector, source and quadrupole temperatures were 250, 280 and 150 °C, respectively. The GC oven was programmed as follow: 75 °C held for 2 min, increased to 120 °C at 40 °C min−1, 120 °C held for 5 min, increased to 320 °C at 20 °C min−1 and held at 320 °C for 7 min.

Supplementary Results

Alterations in gastrointestinal transit time influence distal gut microbial diversity

For both PEG and loperamide-treated groups, there was a decrease in alpha diversity measured using a phylogenetic (tree based) method (Supplementary figure 2A,C). Also the inter-individual variation in microbial communities within a group at a specified point of treatment assessed by unweighted UniFrac was lower than the variation observed between groups. (Supplementary figure 2B,D).

Dietary intake in germ-free and humanized mice.

Humanized mice consume significantly higher amount of cellulose-enriched diet compared to standard diet likely due to decreased caloric density in cellulose-enriched diet (which lacks fermentable polysaccharides), whereas GF mice show no difference in daily intake of cellulose-

enriched and standard diet (Supplementary figure 3). There is no significant difference in daily consumption of PS-deficient diet compared to standard diet in either humanized or GF mice (Supplementary figure 3). There is no significant difference in daily consumption of FOS-enriched diet in humanized mice whereas GF mice consume significantly less FOS-enriched diet likely due to diarrhea (Supplementary figure 3).

Carbohydrate content of diet alters gastrointestinal transit time

Conventionally raised Swiss Webster mice showed comparable changes to humanized mice in GI transit time upon changing from a standard diet to a cellulose-enriched diet, PSD diet or FOS-enriched diet. (Supplementary figure 4).

Carbohydrate content of diet alters distal gut microbial diversity

The microbiota from humanized mice fed cellulose, PSD, or FOS diet show decreased α diversity (as measured by the PD whole tree index) when compared to the microbiota from samples collected from these mice on the standard diet prior to diet switch (Supplementary figure 5A,C,E). The inter-individual variation in microbial communities within a group assessed by unweighted UniFrac is lower than the variation observed between groups, indicating that these changes are robust (Supplementary figure 5B,D,F). The decreased microbial diversity associated with the PSD, cellulose, and FOS diets is generally consistent with the effects of PEG and loperamide.

Microbiota induced change in gastrointestinal transit may be related to gut serotonergic pathway

In order to further address the role of serotonin in microbiota related changes in GI transit time we treated four humanized mice and four germ free mice with SDZ205-557 ($5HT_{3/4}$ antagonist; intra-peritoneal administration) and then measured GI transit 30 minutes following the treatment. The SDZ205-557 treatment led to significant delay in GI transit time in humanized mice (289±13

minutes vs 217±9 minutes; n=4; $p<0.05$) but not in germ free mice (367±9 minutes vs 352±9 minutes; n=4; $p>0.05$) (Supplementary figure 6). These data are consistent with findings reported previously for conventional mice.[3]

Supplementary figure 1. Gastrointestinal transit and polysaccharide content of diet impacts distal gut microbial composition. A. Weighted UniFrac-based PCoA plot (2D representation of 3D plot) of gut microbial communities in humanized mice shows pre-treatment samples cluster together and post-treatment samples cluster based on treatment with PEG or loperamide. B,C,D. Weighted UniFrac-based PCoA plot (2D representation of 3D plot) of gut microbial communities in humanized mice before and during administration of a cellulose-enriched diet (B), PS-deficient diet (C), or a FOS-enriched diet (D) show fecal samples cluster by diet.

Supplementary figure 2. Gut microbiota alpha diversity (PD whole tree) and UniFrac distance boxplots for PEG or loperamide treated mice. A, C. PEG (A) or loperamide (C) treatment results in decreased alpha diversity shown as rarefaction curves using the phylogenetic (tree based) measure of alpha diversity, PD whole tree. B, D. The difference in Unweighted UniFrac distances is greater among samples collected before versus during PEG (B) or loperamide (D) treatment relative to within-group comparisons. "+" represents values outside the interquartile range.

Supplementary figure 3. Dietary intake in germ-free and humanized mice. A. Increased consumption of a cellulose-enriched diet versus a standard diet in humanized mice is likely due to decreased caloric density in cellulose-enriched diet (which lacks fermentable polysaccharides). GF mice show no difference in daily intake of cellulose-enriched and standard diet. B. No significant difference in daily consumption of PS-deficient diet versus standard diet in either humanized or GF mice. C. Humanized mice do not show any significant difference in

daily intake of regular versus FOS-enriched diet. GF mice consume significantly less FOS-enriched diet versus regular diet, likely due to diarrhea in GF mice fed a FOS-diet. *, $p<0.05$.

Supplementary figure 4. Alterations in carbohydrate content of diet alters gastrointestinal transit time. A. Whole gut transit time in conventional mice fed either a cellulose-enriched diet (A), a PS-deficient diet (B), or a FOS-enriched diet (C) compared to standard diet controls. *, $p<0.05$ paired ttest.

Supplementary figure 5. Gut microbiota alpha diversity (PD whole tree) and UniFrac distance boxplots for dietary manipulations of humanized mice. A, C, E. Cellulose-enriched (A), FOS-enriched (C), or PS-deficient (E) diet results in a decrease in alpha diversity shown as rarefaction curves using the phylogenetic (tree based) measure of alpha diversity, PD whole tree. B, D, F. The difference in Unweighted UniFrac distances is greater among samples collected before versus during feeding with cellulose (B), FOS (D), or a PS-deficient diet (F) relative to within-group comparisons.

Supplementary figure 6. Differential effect of 5HT antagonist on gastrointestinal transit time in GF and humanized mice. Whole gut transit time in GF and humanized mice before and after treatment with SDZ205-507 (20mg/kg ip)

Supplementary table 1. Composition of custom diets.

Ingredient (gm/kg)	Polysaccharide deficient chow	Cellulose chow	FOS chow
Casein	200.000	200.000	200.000
cystine	3.000	3.000	3.000
vitamin mix	10.000	10.000	10.000
choline bit	2.500	2.500	2.500
salt mix	35.000	35.000	35.000
Cellulose		339.500	
Fructo-oligosaccharide			100.000
D-glucose	679.000	169.750	579.000
D-fructose		169.750	
Soluble fiber	0.000	0.000	0.000
tbhq	0.014	0.014	0.014
soybean oil	70.000	70.000	70.000
Total (gm)	1000	1000	1000

Supplementary table 2. Significant differences ($p<0.05$ ANOVA with FDR 5%) at the different taxonomic levels between microbial communities from mice on standard diet (Pre-PEG) and standard diet and PEG in water (PEG).

Taxonomic unit	Probability	FDR_corrected	Pre-PEG	PEG	Consensus Lineage
Class					
	0.003	0.037	0.002	0.000	Bacteria; Tenericutes; Mollicutes
Order					
	0.003	0.043	0.002	0.000	Bacteria; Tenericutes; Mollicutes; Anaeroplasmatales
Family					
	0.000	0.000	0.005	0.000	Bacteria; Firmicutes; Clostridia; Clostridiales; Peptococcaceae
	0.001	0.011	0.011	0.103	Bacteria; Firmicutes; Clostridia; Clostridiales; Peptostreptococcaceae
	0.004	0.026	0.192	0.548	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Bacteroidaceae
	0.036	0.099	0.104	0.004	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Porphyromonadaceae
	0.005	0.027	0.001	0.000	Bacteria; Firmicutes; Clostridia; Clostridiales; Eubacteriaceae
	0.003	0.028	0.002	0.000	Bacteria; Tenericutes; Mollicutes; Anaeroplasmatales; Anaeroplasmataceae
Genus					
	0.002	0.020	0.001	0.000	Bacteria; Firmicutes; Clostridia; Clostridiales; Eubacteriaceae; Eubacterium

	0.000	0.004	0.013	0.000	Bacteria; Firmicutes; Erysipelotrichi; Erysipelotrichales; Erysipelotrichaceae; Turicibacter
	0.001	0.011	0.010	0.097	Bacteria; Firmicutes; Clostridia; Clostridiales; Peptostreptococcaceae; Sporacetigenium
	0.000	0.005	0.004	0.000	Bacteria; Firmicutes; Clostridia; Clostridiales; Peptococcaceae; Peptococcus
	0.004	0.027	0.192	0.548	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Bacteroidaceae; Bacteroides
	0.003	0.026	0.002	0.000	Bacteria; Tenericutes; Mollicutes; Anaeroplasmatales; Anaeroplasmataceae; Anaeroplasma

Supplementary table 3. Significant differences ($p<0.05$ ANOVA with FDR 5%) at different taxonomic levels between microbial communities from mice fed a standard diet (Pre-loperamide) versus a standard diet plus loperamide in water (Loperamide).

Taxonomic unit	Probability	FDR_corrected	Pre-loperamide	Loperamide	Consensus Lineage
Phyllum					
	0.026	0.032	0.000	0.002	Bacteria; Verrucomicrobia
	0.001	0.003	0.659	0.368	Bacteria; Firmicutes
	0.001	0.002	0.307	0.560	Bacteria; Bacteroidetes
	0.001	0.002	0.029	0.056	Bacteria; Other
	0.028	0.028	0.003	0.015	Bacteria; Proteobacteria
Class					
	0.015	0.033	0.003	0.012	Bacteria; Proteobacteria; Betaproteobacteria
	0.026	0.046	0.000	0.002	Bacteria; Verrucomicrobia; Verrucomicrobiae
	0.061	0.092	0.004	0.013	Bacteria; Bacteroidetes; Other
	0.001	0.008	0.303	0.546	Bacteria; Bacteroidetes; Bacteroidia
	0.001	0.006	0.571	0.258	Bacteria; Firmicutes; Clostridia
Order					
	0.001	0.005	0.029	0.056	Bacteria; Other; Other; Other
	0.001	0.009	0.303	0.546	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales
	0.001	0.007	0.570	0.254	Bacteria; Firmicutes; Clostridia; Clostridiales
Family					
	0.001	0.025	0.029	0.056	Bacteria; Other; Other; Other;

					Other
	0.002	0.022	0.409	0.104	Bacteria; Firmicutes; Clostridia; Clostridiales; Lachnospiraceae
Genus					
	0.001	0.019	0.029	0.056	Bacteria; Other; Other; Other; Other; Other

Supplementary table 4. Significant differences ($p<0.05$ ANOVA with FDR 5%) at different taxonomic levels between microbial communities from mice on standard diet (Pre-cellulose) versus a cellulose-enriched diet (Cellulose).

Taxonomic unit	Probability	FDR_corrected	Pre-cellulose	Cellulose	Consensus Lineage
Class					
	0.004	0.044	0.005	0.095	Bacteria; Firmicutes; Other
Order					
	0.004	0.044	0.005	0.095	Bacteria; Firmicutes; Other; Other
Family					
	0.004	0.043	0.261	0.060	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Porphyromonadaceae
	0.006	0.043	0.203	0.531	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Bacteroidaceae
Genus					
	0.006	0.045	0.203	0.531	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Bacteroidaceae; Bacteroides

Supplementary table 5. Significantly different ($p<0.01$; unpaired ttest and fold change >100) masses for fecal metabolites between mice on standard diet versus FOS-enriched diet.

Regular>FOS				FOS>Regular		
Mass	Fold Change	p.value		Mass	Fold Change	pvalue
307.1223	0.007825	5.49E-08		363.2114	6.8307	0.000506
477.2717	0.004224	4.17E-07		290.1965	6.9806	0.001552
162.0547	0.007262	4.18E-07		467.2947	7.0184	4.56E-05
541.3393	0.008595	1.09E-06		476.2689	7.0952	7.23E-05
208.0602	0.00596	2.05E-06		328.2226	7.1057	0.005244
192.0654	0.003591	2.90E-06		272.1498	7.2815	2.40E-06
209.0635	0.00205	5.17E-06		491.2428	7.2945	4.53E-05
344.2801	0.003958	6.84E-06		262.1652	7.3066	0.002947
214.0474	0.000491	6.96E-06		523.2564	7.35	0.000279
231.0453	0.000339	7.81E-06		378.203	7.3626	0.008347
578.3309	0.007522	7.83E-06		327.2533	7.3918	0.004231
230.0421	0.000255	7.84E-06		462.2893	7.5415	2.80E-06
215.0508	0.008788	9.33E-06		524.2675	7.6389	1.20E-06
146.06	0.002189	1.12E-05		150.0585	7.6666	0.000138
308.2589	0.0011	2.13E-05		414.25	7.6803	2.57E-05
497.3037	0.004262	3.01E-05		484.3029	7.7035	1.07E-05
496.3003	0.00845	5.86E-05		283.1072	7.9326	7.15E-05
786.5959	0.006067	7.00E-05		448.1989	8.0479	0.002647
247.5469	0.000727	8.60E-05		312.1422	8.404	2.60E-05
785.5926	0.005968	8.95E-05		504.2817	8.5652	0.001516
213.6118	0.00136	0.000101		312.1789	8.7181	0.007774
226.0627	0.002592	0.000135		503.2433	8.7299	0.004664
380.2988	0.009832	0.000142		214.1439	8.7647	0.000237
437.0954	0.000475	0.000207		360.2028	8.8569	0.001977
353.1016	0.000575	0.000242		320.2952	9.1365	0.005988
164.5338	0.000759	0.000244		358.2245	9.338	0.005868
348.1449	0.001944	0.00025		410.2518	9.6303	0.001146
473.2404	0.001004	0.000306		248.1283	9.6397	0.001529
309.0874	0.007454	0.000364		280.1523	9.7191	0.000404
443.2298	0.00151	0.000477		362.2081	9.7207	0.000429
474.244	0.002267	0.000494		264.1234	9.9197	0.000939
248.1519	0.003687	0.000504		362.2543	9.9382	0.000747
421.0709	0.001238	0.00057		466.2922	9.9955	0.000154
135.117	0.00805	0.000579		292.1758	10.09	0.001693
453.0608	0.000984	0.000644		230.1178	10.501	0.000552

246.0738	0.003628	0.000784		426.2165	10.886	0.000899
549.2932	0.002724	0.000902				
525.2679	0.00197	0.000906				
246.0634	0.004074	0.000958				
303.1806	0.006827	0.000989				
427.0889	0.002045	0.002025				
252.0243	0.001633	0.005107				
255.137	0.003536	0.00839				

Supplementary References

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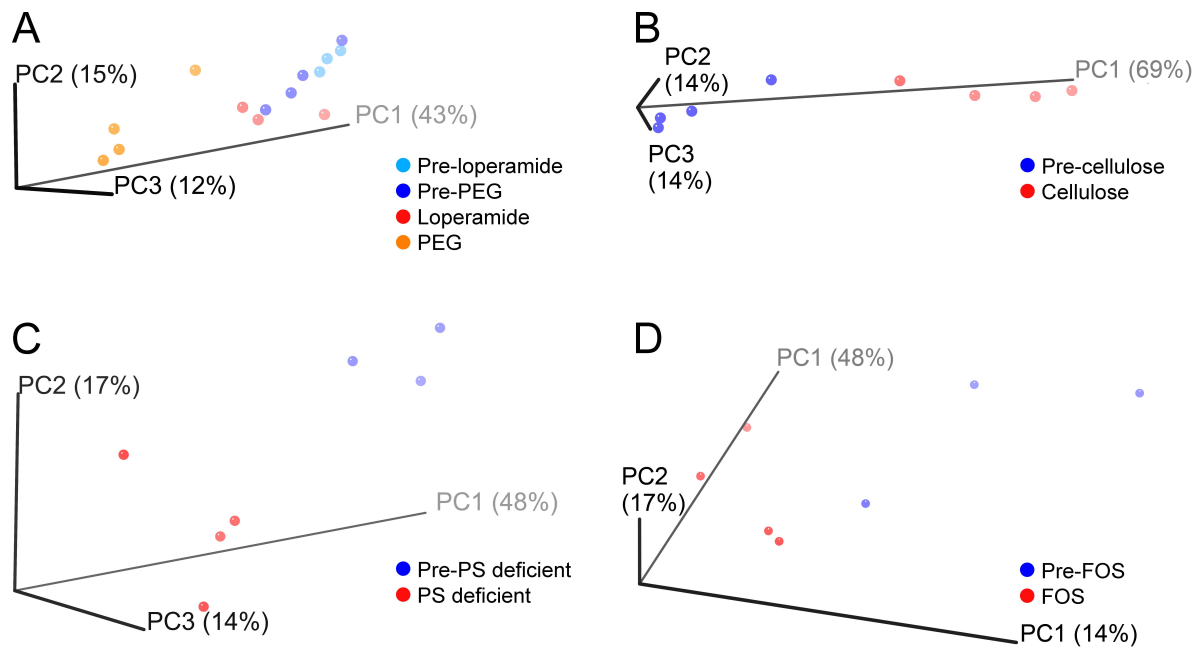
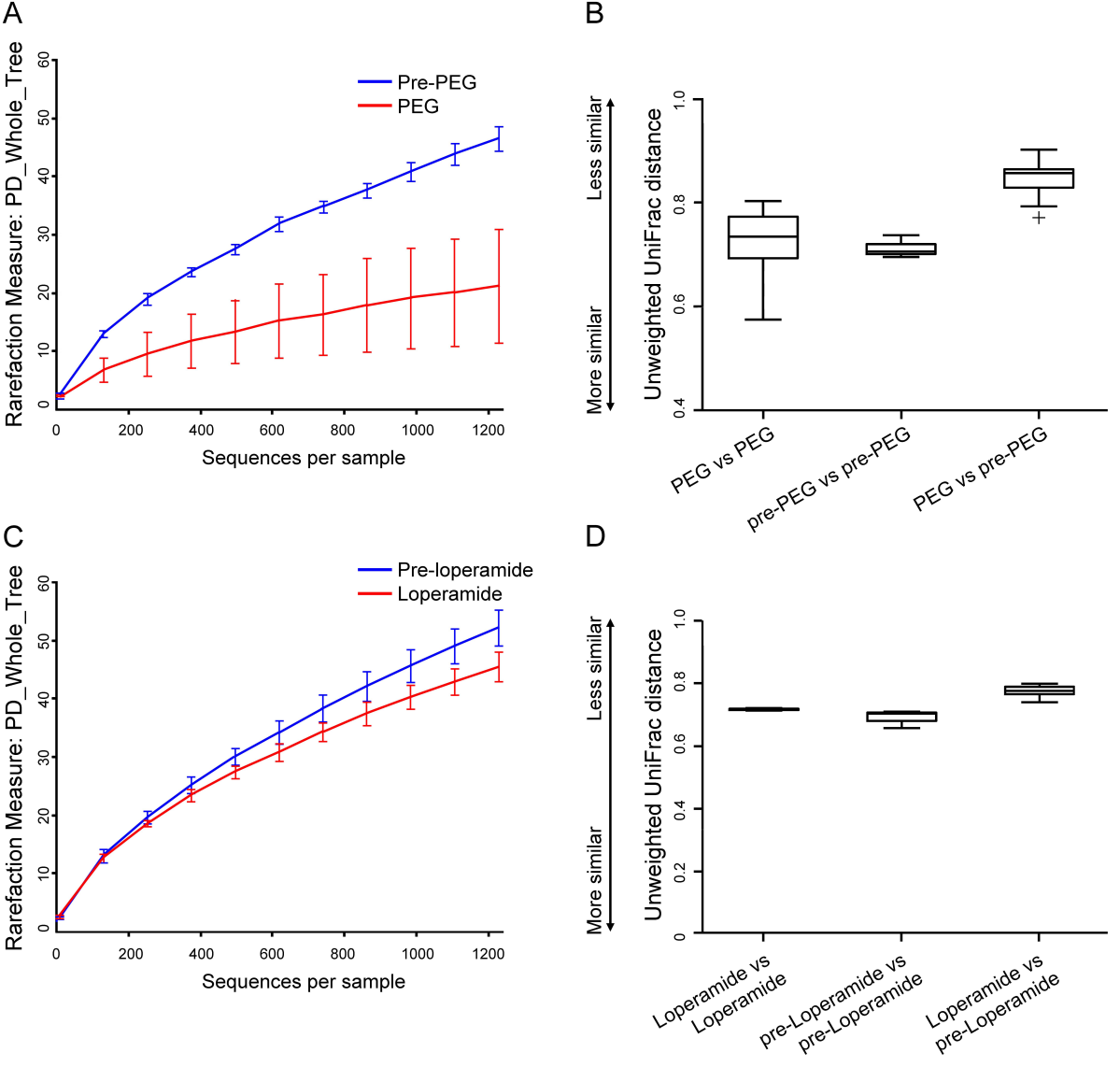
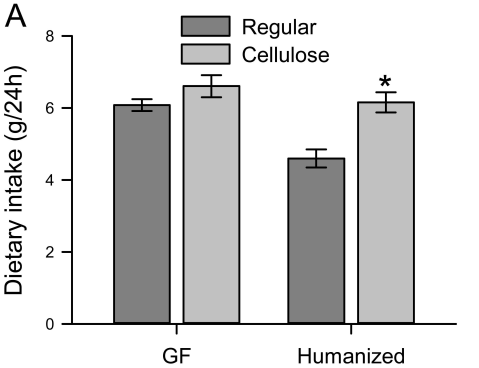
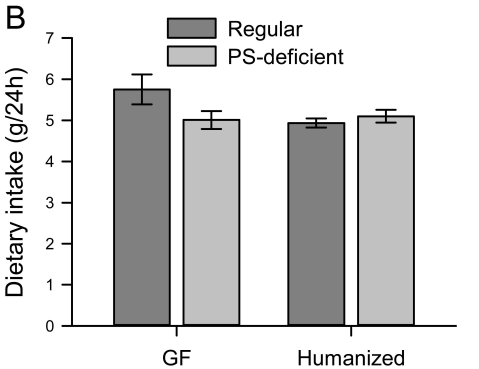


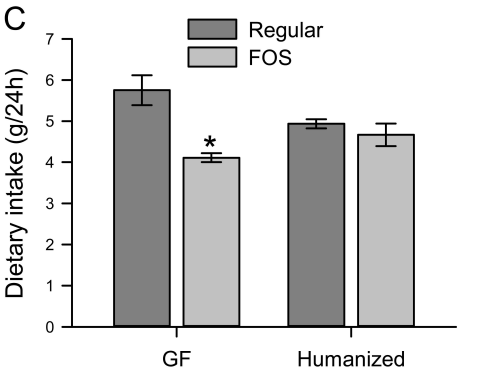
Supplementary figure 1



Supplementary figure 2







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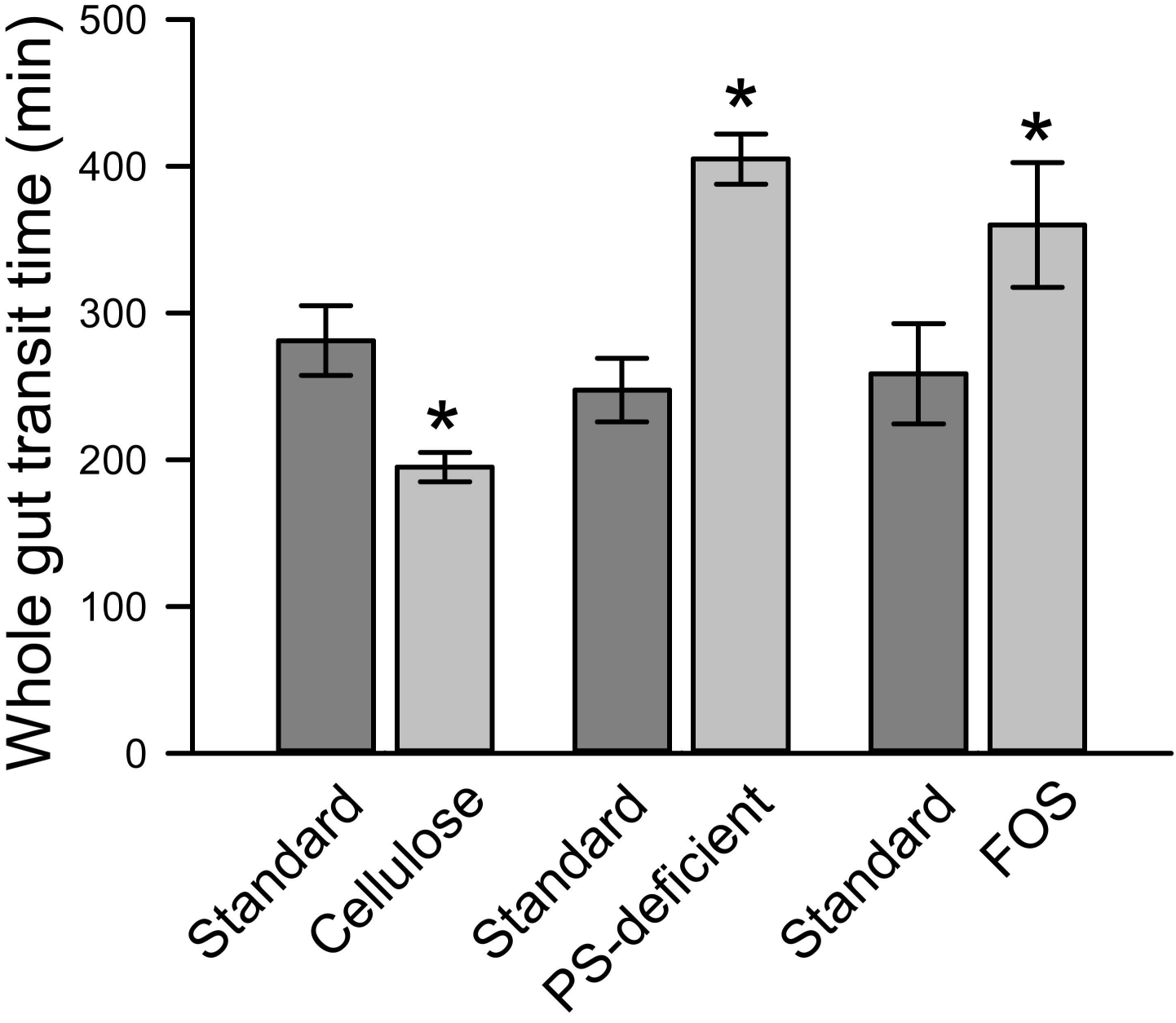

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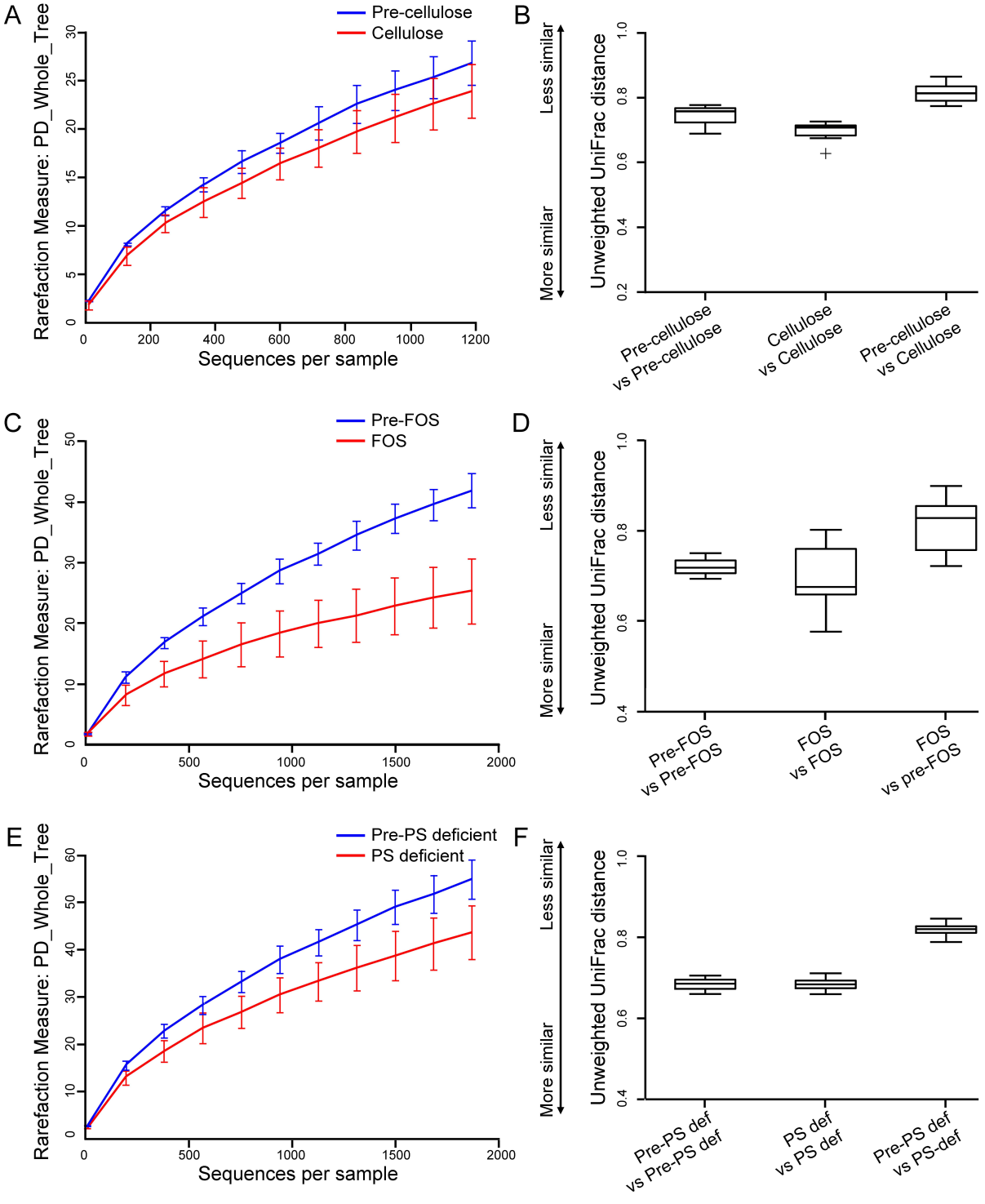
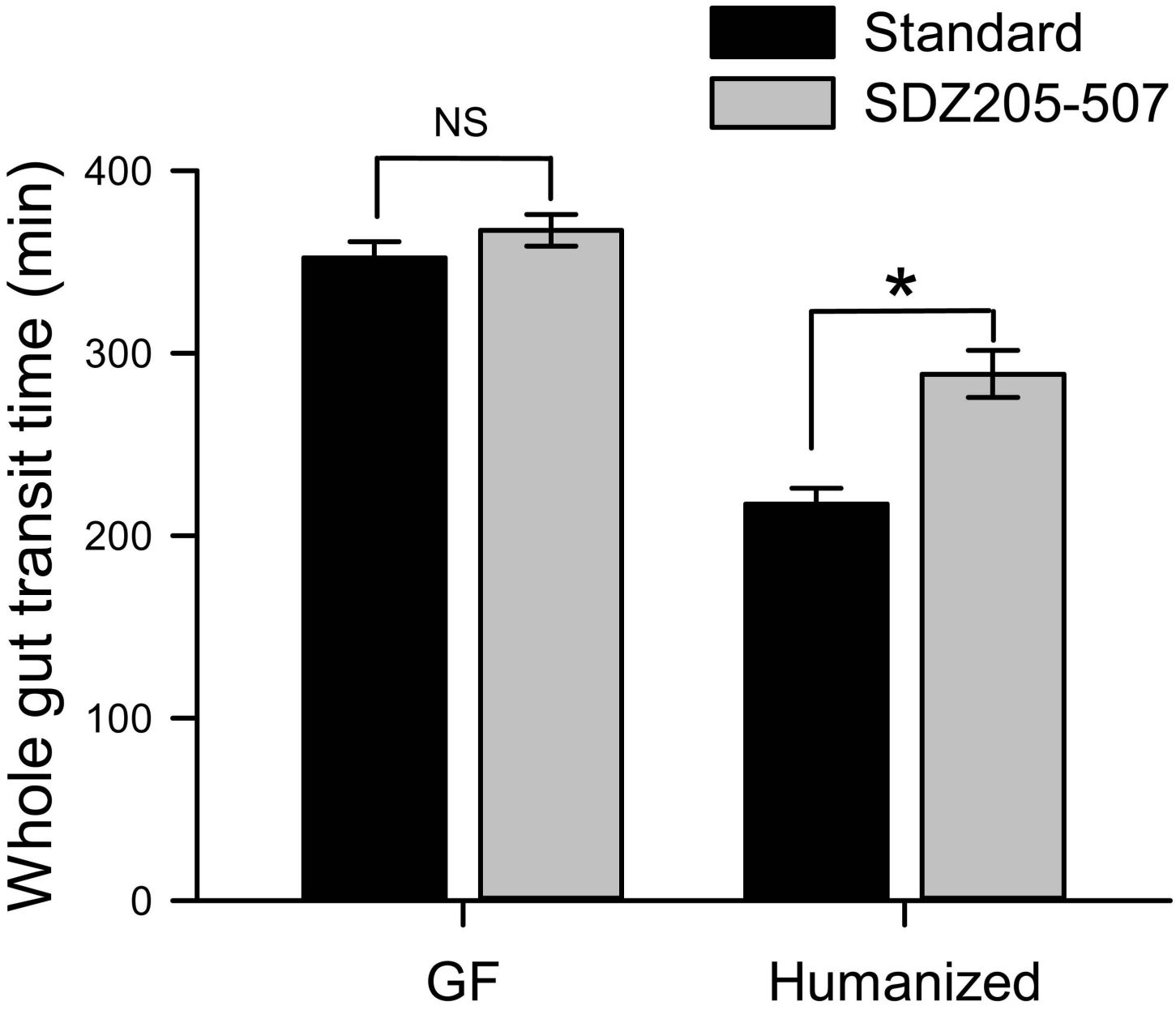


Supplementary figure 5



Supplementary figure 6