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Supplemental information

Lower brown adipose tissue activity is associated with non-alcoholic fatty liver disease but not changes in the gut microbiota

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SUPPLEMENTAL INFORMATION

Table S1. Participant and study characteristics between brown adipose tissue groups. (Related to Table 1).

	Low BAT Activity (n=33)	**High BAT activity (n=27)**	***p***
Age (years)	28.3 [24.8, 41.4]	23.9 [22.3, 30.6]	0.012†
Gender (male)	16 (48%)	21 (78%)	0.020$
Weight (kg)	85.2 (21.1)	77.8 (14.4)	0.111
Waist circumference (cm)	95.9 (17.0)	83.6 (14.4)	0.004*
BMI (kg/m^2)	30.2 (7.5)	25.1 (4.7)	0.002*
Body Fat (%) [a]	36.6 (11.2)	23.4 (10.4)	<0.001*
Hepatic fat (%) [b]	5.8 [5.0, 8.1]	5.2 [4.4, 6.3]	0.054
Absolute cold-induced decline in SCV PDFF (%)	1.2 (1.2)	5.1 (1.7)	<0.001*
Cold-induced percent decline in SCV PDFF (%)	1.7 (1.6)	7.8 (3.1)	<0.001*
VAT (cm^2) [c]	44.6 [15.0, 64.4]	8.5 [5.5, 23.3]	<0.001†
SAT (cm^2) [c]	121.4 (70.0)	73.8 (57.4)	0.008*
Fasting plasma glucose (mmol/L)	4.8 [4.5, 5.1]	4.6 [4.1, 4.8]	0.020†
2hr glucose (mmol/L) [d]	5.4 (2.0)	4.2 (1.1)	0.049*
HbA1c (%)	5.3 [4.9, 5.6]	5.1 [4.9, 5.3]	0.171
Total cholesterol (mmol/L)	4.4 (0.8)	4.3 (0.7)	0.490
Triglycerides (mmol/L)	0.9 [0.7, 1.6]	0.7 [0.6, 1.0]	0.078
HDL-C (mmol/L)	1.3 (0.3)	1.4 (0.3)	0.302
LDL-C (mmol/L)	2.6 (0.6)	2.5 (0.7)	0.616
Non-HDL-C (mmol/L)	3.2 (0.8)	2.9 (0.8)	0.263
Systolic blood pressure (mmHg)	113.5 (12.7)	108.7 (11.0)	0.130
Diastolic blood pressure (mmHg)	75.0 (9.3)	73.6 (10.5)	0.586
AST (U/L)	17.0 [15.5, 20.0]	21.0 [17.0, 23.0]	0.037†
ALT (U/L)	21.0 [16.0, 28.5]	19.0 [15.0, 25.0]	0.430
GGT (U/L)	20.0 [14.0, 33.5]	16.0 [13.0, 20.0]	0.108
Outdoor temperature 1hr before visit 2 (°C)	7.8 (10.3)	6.4 (9.7)	0.595
Muscles MVC (%) [e]	2.0 [1.1, 2.9]	2.0 [0.8, 2.9]	0.562
$\Delta_{\text{Outlet-inlet}}$ of the suit (°C) [F]	1.5 (0.2)	1.6 (0.2)	0.309

Values are presented as n (%) for categorical variables and continuous variables are presented as mean (SD) for normally distributed variables or median [IQ1, IQ3] for non-normally distributed variables. * - $p < 0.05$ via independent sample t-test; † - $p < 0.05$ via Mann-Whitney U test; $ via Chi-Square test. [a] one participant was above the weight threshold for body composition analysis via DEXA. [b] no liver scan for one participant as they did not fit inside the scanner. [c] no abdominal volume data for four participants (n=2: acquisition error; n=1: did not fit inside the scanner; n=1: motion issues). [d] 2hr glucose was not performed in 29 participants (n=2: fainted during their baseline bloodwork; n=1: known type 2 diabetic; n=26: recalled glucose solution). [e] Motion artifacts for one participant. [F] No outlet-inlet data for five participants (n=4: data were not acquired, n= 1: an error in the data acquisition). BAT; brown adipose tissue, BMI; body mass index, SCV PDFF; supraclavicular proton density fat fraction, VAT; visceral adipose tissue, SAT; subcutaneous adipose tissue, HbA1c; hemoglobin A1c, HDL-C; high-density lipoprotein cholesterol, LDL-C; low-density lipoprotein cholesterol, Non-HDL-C; non-high-density lipoprotein cholesterol, AST; aspartate aminotransferase, ALT; alanine aminotransferase, GGT; gamma-glutamyl transferase, MVC; maximal voluntary contraction.

Table S2. Macronutrient and dietary fibre intake 24 hours prior to measurement of BAT activity. (Related to Table 1).

	Normal liver fat (n=30)	**Hepatic Steatosis (n=29)**	***p***
Weight (kg)	70.7 (13.6)	91.7 (14.8)	< 0.001 *
Total calories (kcal)	2274.65 (712.6)	2384.63 (1026.4)	0.633
Total kcal (related to weight)	33.22 (11.8)	27.09 (13.2)	0.065
Protein			
Protein (kcal)	372.60 (135.9)	453.93 (245.4)	0.119
Calories from protein (%)	17.69 (8.7)	19.66 (8.4)	0.382
Fat			
Fat (kcal)	797.50 (437.8)	811.49 (376.7)	0.896
Calories from fat (%)	34.10 (13.9)	35.49 (11.4)	0.677
Carbohydrate			
Carbohydrate (kcal)	1117.39 (488.7)	1133.99 (713.7)	0.917
Calories from carbohydrate (%)	48.90 (14.7)	45.61 (14.5)	0.391
Fibre (g)	23.12 (14.2)	22.59 (17.7)	0.899
Values are presented as mean (SD). * - $p < 0.05$ via independent sample t-test.			

Table S3. Hierarchical regression for alpha diversity indices in relation to cold-induced percent decline in supraclavicular proton density fat fraction, between BAT and between NAFLD groups. (Related to Figures 3 and S3).

Alpha diversity indices in relation to cold-induced percent decline in SCV PDFF								
	Shannon				**Inverse Simpson**			
	B	SE	T-Statistic	*p*	B	SE	T- Statistic	*p*
Model 1								
Percent decline in SCV PDFF (%)	0.011	0.014	0.781	0.438	0.107	0.273	0.392	0.697
Model 2								
Age (years)	0.001	0.006	0.195	0.846	-0.035	0.119	-0.291	0.772
Percent decline in SCV PDFF (%)	0.012	0.015	0.783	0.437	0.069	0.304	0.226	0.822
Model 3								
Age (years)	0.002	0.006	0.380	0.705	-0.007	0.125	-0.056	0.956
Body Fat	-0.004	0.006	-0.640	0.525	-0.080	0.111	-0.719	0.475
Percent decline in SCV PDFF (%)	0.006	0.018	0.330	0.743	-0.066	0.359	-0.183	0.855
Alpha diversity indices between BAT groups								
	Shannon				**Inverse Simpson**			
	B	SE	T-Statistic	*p*	B	SE	T- Statistic	*p*
Model 1								
High BAT/ Low BAT	-0.008	0.103	-0.079	0.937	-1.186	2.058	-0.576	0.567
Model 2								
Age (years)	-0.001	0.006	-0.189	0.851	-0.072	0.113	-0.642	0.523
High BAT/ Low BAT	-0.015	0.109	-0.133	0.894	-1.622	2.178	-0.745	0.459
Model 3								
Age (years)	0.002	0.006	0.274	0.785	-0.014	0.122	-0.118	0.907
Body Fat	-0.006	0.005	-1.136	0.261	-0.126	0.105	-1.205	0.234
High BAT/ Low BAT	-0.077	0.122	-0.630	0.531	-2.937	2.428	-1.210	0.232
Alpha diversity indices between NAFLD groups								
	Shannon				**Inverse Simpson**			
	B	SE	T-Statistic	*p*	B	SE	T- Statistic	*p*
Model 1								
NAFLD positive/negative	-0.232	0.098	-2.36	0.022*	-4.283	1.977	-2.166	0.034*
Model 2								
Age (years)	0.006	0.006	1.04	0.303	0.071	0.116	0.614	0.542
NAFLD positive/negative	-0.284	0.110	-2.58	0.013*	-4.916	2.240	-2.195	0.032*
Model 3								
Age (years)	0.006	0.006	0.964	0.339	0.066	0.123	0.533	0.596
Body Fat	0.000	0.005	0.049	0.961	0.014	0.100	0.143	0.887
NAFLD positive/negative	-0.287	0.122	-2.351	0.022*	-5.061	2.475	-2.044	0.046*

Model 1, without adjusting to confounding variables, Model 2; controlling for age and Model 3, controlling for age and body fat percentage. * - $p < 0.05$. SCV PDFF; supraclavicular proton density fat fraction, BAT; brown adipose tissue, Low BAT; low BAT thermogenesis; High BAT; high BAT thermogenesis, NAFLD; non-acholic fatty liver disease, NAFLD positive; with hepatic steatosis, NAFLD negative; with normal hepatic fat.

Table S4. Differential gene expression analysis at the genus level. (Related to Figures 3 and S3).

Cold-induced percent decline in supraclavicular proton density fat fraction									
	Model 1			**Model 2**			**Model 3**		
Genera	**LFC**	***p* value**	***p* adj**	**LFC**	***P* value**	***p* adj**	**LFC**	***p* value**	***p* adj**
Catenisphaera	-2.94	0.045	0.515	-9.26	1.3E-08	1.7E-06*	1.04	0.584	0.990
Coriobacteriaceae _UCG-003	- 2.80	0.056	0.523	-8.96	3.7E-08	2.5E-06*	0.984	0.606	0.990
Libanicoccus	-2.54	0.085	0.523	-8.29	3.5E-07	1.6E-05*	0.893	0.641	0.990
Brown adipose tissue (BAT) groups									
Succiniclasticum	0.00	1.00	NA	5.12	0.093	0.593	-26.39	1.9E-14	2.5E-12*
Non-acholic fatty liver disease (NAFLD) groups									
Catenisphaera	0	1	NA	-7.25	0.027	0.246	-30.0	9.6E-17	4.4E-15*
Coriobacteriaceae_UCG-003	0	1	NA	-6.89	0.036	0.280	-30.0	9.6E-17	4.4E-15*
Libanicoccus	0	1	NA	-6.21	0.058	0.393	-30.0	9.7E-17	4.4E-15*
f_Muribaculaceae	0.68	0.817	NA	7.52	0.021	0.223	12.08	0.001	0.026*
Catenibacterium	8.38	5.2E-06	0.0004*	0.531	0.824	0.942	0.688	0.796	0.964
Dorea	1.35	1.7E-05	0.001*	1.36	0.0001	0.017*	1.15	0.003	0.058
Christensenellaceae_R-7_group	-2.62	9.6E-05	0.002*	-1.78	0.039	0.282	-1.58	0.088	0.478
Lachnospira	-1.69	0.0002	0.003*	-1.79	0.001	0.039*	-1.83	0.002	0.055
Blautia	1.00	0.0003	0.005*	0.99	0.002	0.059	0.834	0.017	0.187
Bacteroides	-1.60	0.001	0.011*	-1.50	0.008	0.170	-1.44	0.020	0.208
Barnesiella	-3.97	0.002	0.016*	-3.17	0.034	0.280	-3.07	0.058	0.377
Lactococcus	2.68	0.003	0.030*	2.59	0.017	0.216	2.74	0.024	0.228
Unknown genus for Lachnospiraceae family	0.56	0.005	0.042*	0.190	0.373	0.758	0.235	0.323	0.785

Alistipes	-1.85	0.006	0.042*	-1.36	0.095	0.446	-1.40	0.119	0.520
Tyzzerella_4	1.41	0.008	0.049*	1.12	0.068	0.403	0.99	0.145	0.520
Phascolarctobacterium	-2.62	0.008	0.049*	-1.93	0.098	0.446	-2.24	0.084	0.475
Unknown genus for Rhodospirillales order	-6.57	0.022	0.091	-8.52	0.0003	0.023*	-6.45	0.015	0.187

LCF; Log2 fold of change, p-value; calculated via Wald test, *p* adj; adjusted *p*-value via Benjamini and Hochberg procedure. * - Adj $p < 0.05$, Model 1, without adjusting to confounding variables, Model 2; controlling for age, and Model 3, controlling for age and body fat percentage. NA: *p*-value was not calculated as DESEq2 was not able to calculate the variance of the taxon.

Table S5. Participant characteristics for donors of stool samples that were used for germ free mice colonization. (Related to Figure 4).

	Donors with high BAT activity			Donors with low BAT activity			
	A	B	C	D	E	F	G
Age (years)	24.7	22.7	22.5	36.6	24.0	49.6	24.8
Gender	Male	Male	Male	Male	Male	Male	Male
Weight (kg)	79.7	81.3	66.6	98.7	88.6	74.6	116.9
BMI (kg/m^2)	22.4	24.9	24.4	30.3	31.7	25.4	36.3
Waist circumference (cm)	84.4	78.5	75	106.6	97.5	85.8	102.7
Body Fat (%)	12.4	17.1	11.7	34	38.4	26.7	47.9
Hepatic fat (%)	5.2	5.5	4.0	18.1	20.2	8.9	7.4
Absolute cold-induced decline in SCV PDFF (%)	7.9	7.0	7.9	-1.2	0.3	0.7	1.5
Cold-induced percent decline in SCV PDFF (%)	12.8	12.3	13.5	-1.5	0.3	0.8	2.1
VAT (cm^2)	8.7	5.5	13.5	60.1	64.6	59.8	22.9
SAT (cm^2)	45.7	45.8	31.1	98.9	169.8	57.4	179.5
Fasting plasma glucose (mmol/L)	4.6	4	3.9	5.1	4.8	9.1	5.7
2h glucose (mmol/L) [a]	3.9	3.8	3.1	7	4.7	-	-
HbA1c (%)	4.9	5.1	5.3	5.6	4.9	8	5.3
Total cholesterol (mmol/L)	4.8	4.2	3.9	5.7	5.5	4.6	3.9
Triglycerides (mmol/L)	1.0	0.7	0.7	3.0	0.8	1.9	0.6
HDL-C (mmol/L)	1.7	1.3	1.4	1.3	1.5	1	1.3
LDL-C (mmol/L)	2.6	2.6	2.1	3.1	3.6	2.7	2.3
Non-HDL-C (mmol/L)	3.1	2.9	2.4	4.5	4.0	3.6	2.5
Systolic blood pressure (mmHg)	104	111	97	118	111	113	115
Diastolic blood pressure (mmHg)	79	74	63	78	75	78	61
AST (U/L)	31	21	16	19	50	15	12
ALT (U/L)	26	15	14	40	94	25	13
GGT (U/L)	17	16	43	37	55	25	20
NAFLD group	NAFLD negative	NAFLD negative	NAFLD negative	NAFLD positive	NAFLD positive	NAFLD positive	NAFLD positive

[a] 2hr glucose was not performed for two participants (F; known type 2 diabetic and G; recalled glucose solution). BAT; brown adipose tissue, BMI; body mass index, SCV PDFF; supraclavicular proton density fat fraction, VAT; visceral adipose tissue, SAT; subcutaneous adipose tissue, HbA1c; hemoglobin A1c, HDL-C; high-density lipoprotein cholesterol, LDL-C; low-density lipoprotein cholesterol, Non-HDL-C; non-high-density lipoprotein cholesterol, AST; aspartate aminotransferase, ALT; alanine aminotransferase, GGT; gamma-glutamyl transferase.

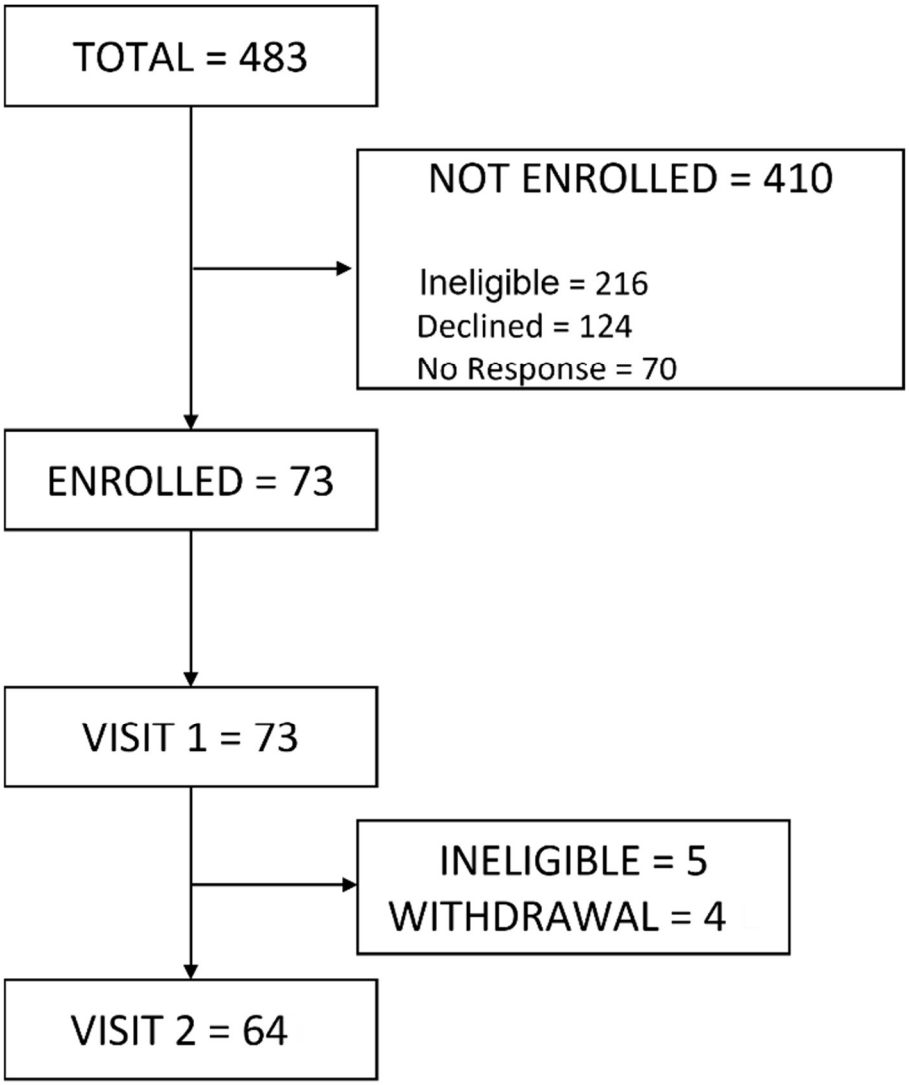


Figure S1. Recruitment Flow Chart. (Related to Table 1).

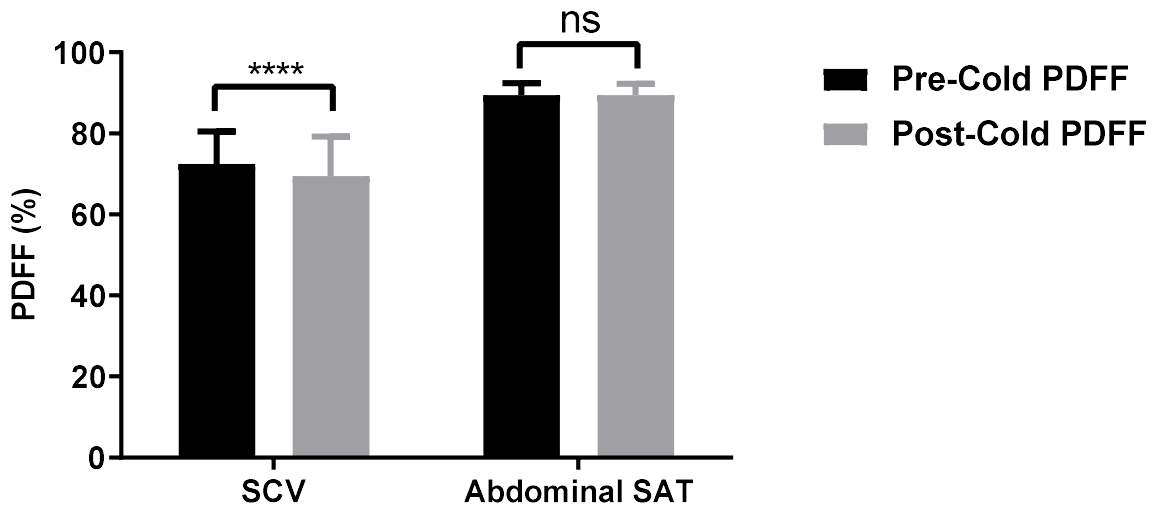


Figure S2. Proton density fat fraction in brown adipose tissue and white adipose tissue before and after cold exposure. (Related to Table 1).

Proton density fat fraction (PDFF) in supraclavicular (SCV) brown adipose tissue (n= 60) and abdominal subcutaneous adipose tissue (SAT) (n=37) before (pre-cold) and after (post-cold) cold exposure. **** - $p < 0.001$ via paired Student's t-test.

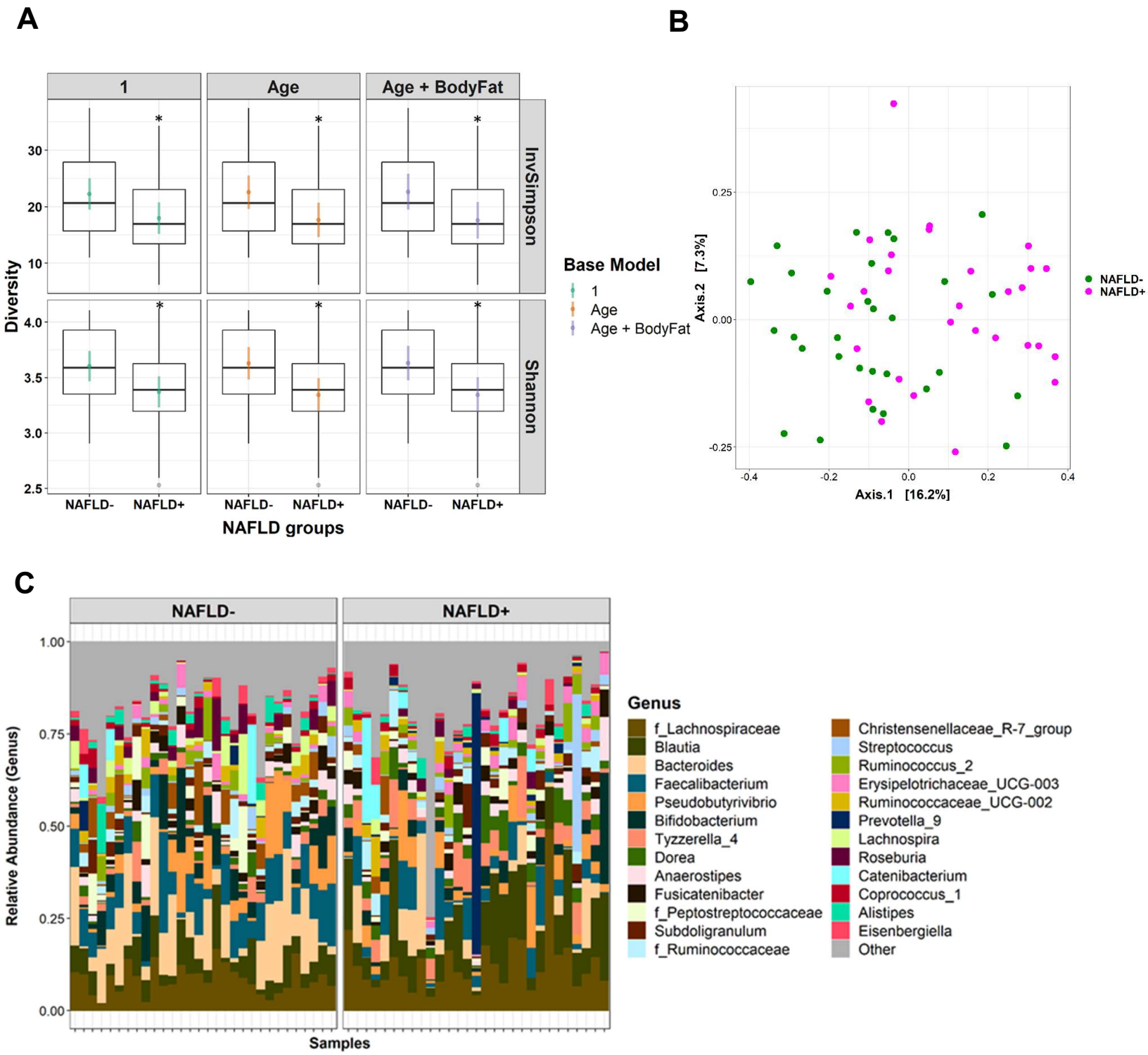
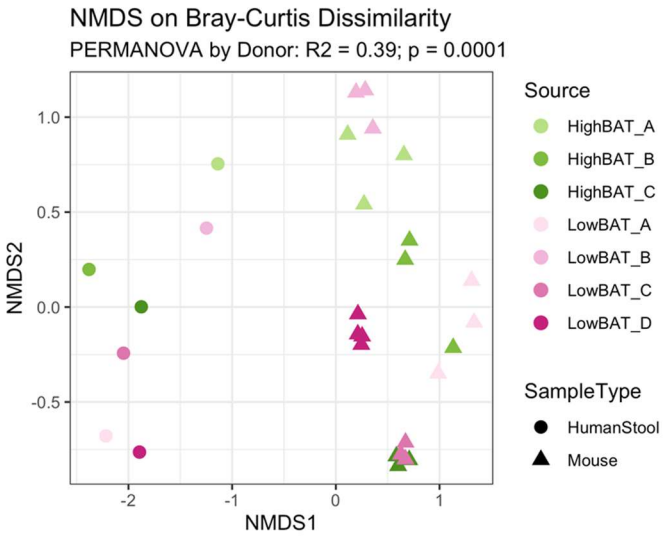


Figure S3. Gut microbiota between NAFLD groups. (Related to Figure 3).

Alpha diversity between NAFLD groups (without and with hepatic steatosis; NAFLD- and NAFLD+ respectively)**.** **(A)** PCoA on Bray-Curtis dissimilarity distances between NAFLD groups. **(B)** Relative abundance of top 25 genera between NAFLD groups. **(C)** For A, data are represented as the median, IQ range, and 95% range of the data. The colored points and lines are the point estimates of the means from the regression models and the 95% confidence intervals of the model estimates of the means, respectively. For B, axes 1 and 2 captured 16.2 and 7.3% in the variation between samples, respectively. * - $p<0.05$.

A



B

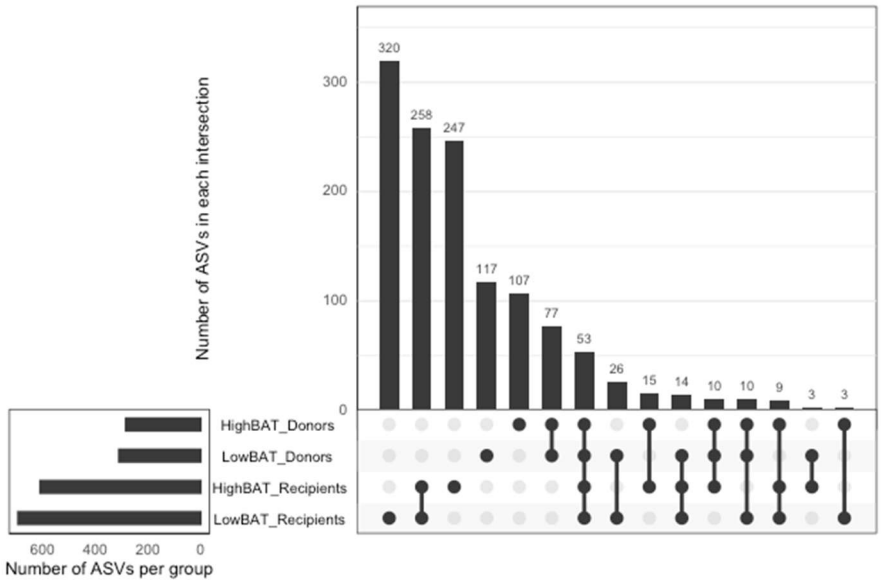


Figure S4. Fecal microbiota transfer from fecal samples of participants with high and low BAT activity to axenic mice. (Related to Figure 4).

The fecal microbiota of fecal transplanted axenic mice and their human fecal sample sources. Each donor and their fecal sample were classified as either High BAT activity (labeled HighBAT_A, HighBAT_B, or HighBAT_C) or Low BAT activity (labeled LowBAT_A, LowBAT_B, LowBAT_C, LowBAT_D). Non-metric multidimensional scaling (NMDS) plot of the Bray-Curtis dissimilarity metric colored by the source of stool to denote BAT activity classification and different donors, and with shapes to denote either the human stool donor or the axenic mice recipients (**A**). Upset plot comparing ASVs present in each group, where the y-axis shows the number of ASVs common between the groups identified along the x-axis. The bar graph beside the x-axis shows the total number of ASVs detected in each group **(B)**.

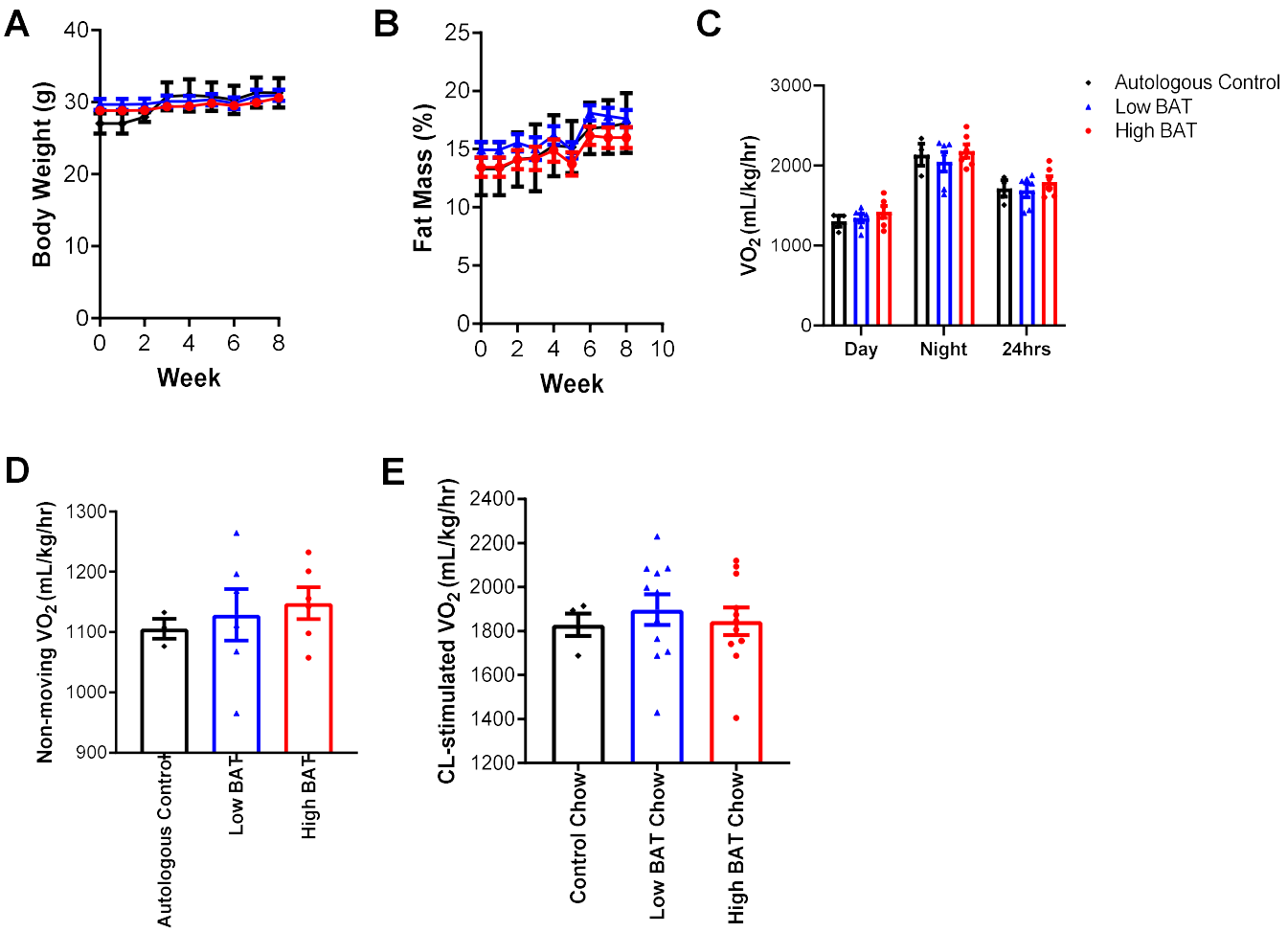


Figure S5: Human brown adipose tissue activity is not transmissible via gut microbes in SPF mice. (Related to Figure 4).

Weekly **(A)** body mass and **(B)** percent fat mass. **(C)** Average oxygen consumption (VO2) during light and dark cycles, and over a 24hr period (average of light and dark), along with **(D)** non-moving VO2 measures after 5 weeks of colonization. Oxygen consumption of anesthetized mice following CL-316,243 administration after 7 weeks of colonization. Data are expressed as means ± SEM. Significance was determined using a One-Way ANOVA and Tukey's multiple comparison test. n=3-12/group.