

Supplementary Materials for

Gut Commensal *Candida albicans* Constrains Whole-Body Energy Storage in Mice

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Figs. S1 to S6

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Other Supplementary Materials for this manuscript include the following:

Source Data S1

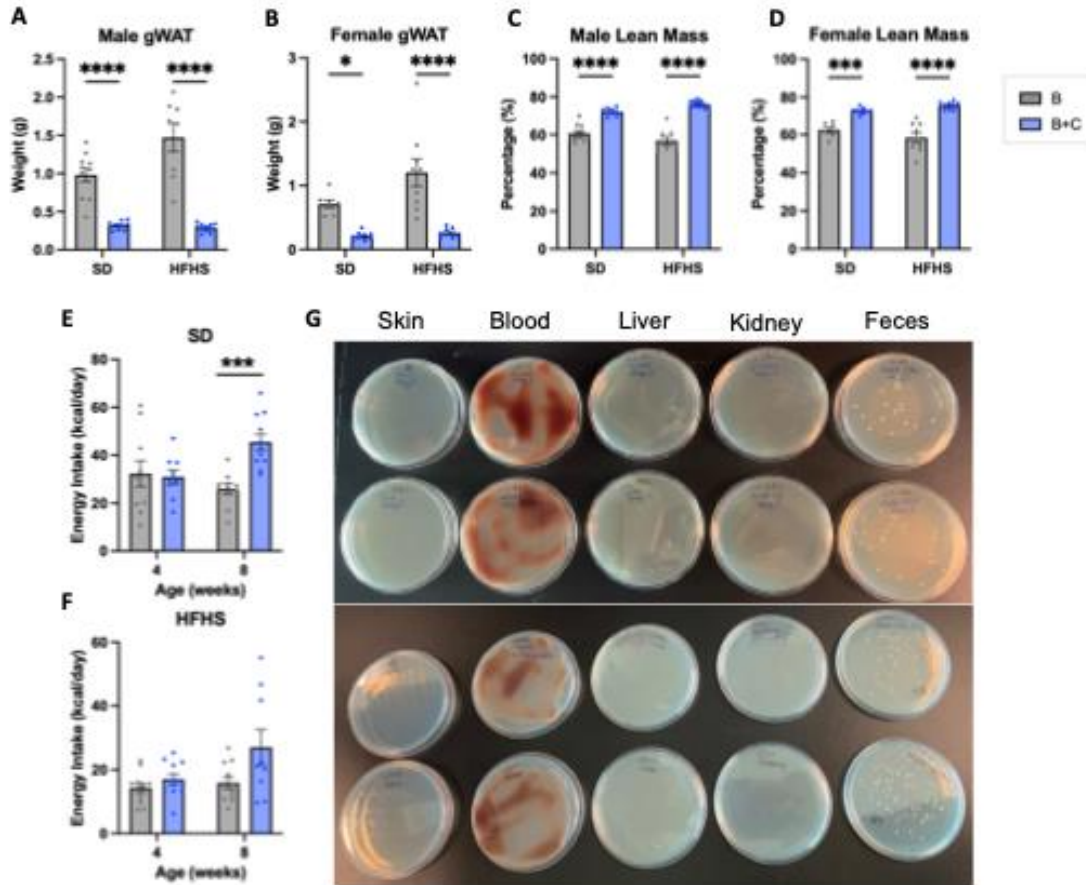


Figure S1. *C. albicans* colonization limits adipose tissue expansion without altering energy intake or disseminating beyond the intestine.

A-B) Gonadal WAT weight and **C-D)** lean mass percentages, in males and females. Statistical significance was determined by two-way ANOVA followed by Šídák's multiple comparisons test. **E-F)** Average daily energy intake of mice fed a standard diet (SD; **E**) or high-fat high-sucrose diet (HFHS; **F**). Each point represents the mean daily energy intake of an individual cage. Normality was assessed using the Shapiro–Wilk test, and comparisons between B and B+C groups at each time point were performed using unpaired *t*-tests. Data represented as mean $\pm$ SEM. Outliers removed with ROUT ($Q = 1\%$). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. **G)** Representative yeast-mold agar culture plates demonstrating recovery of *C. albicans* from feces but not from liver, kidney, blood or skin collected from B+C mice after 48 h of incubation at room temperature.

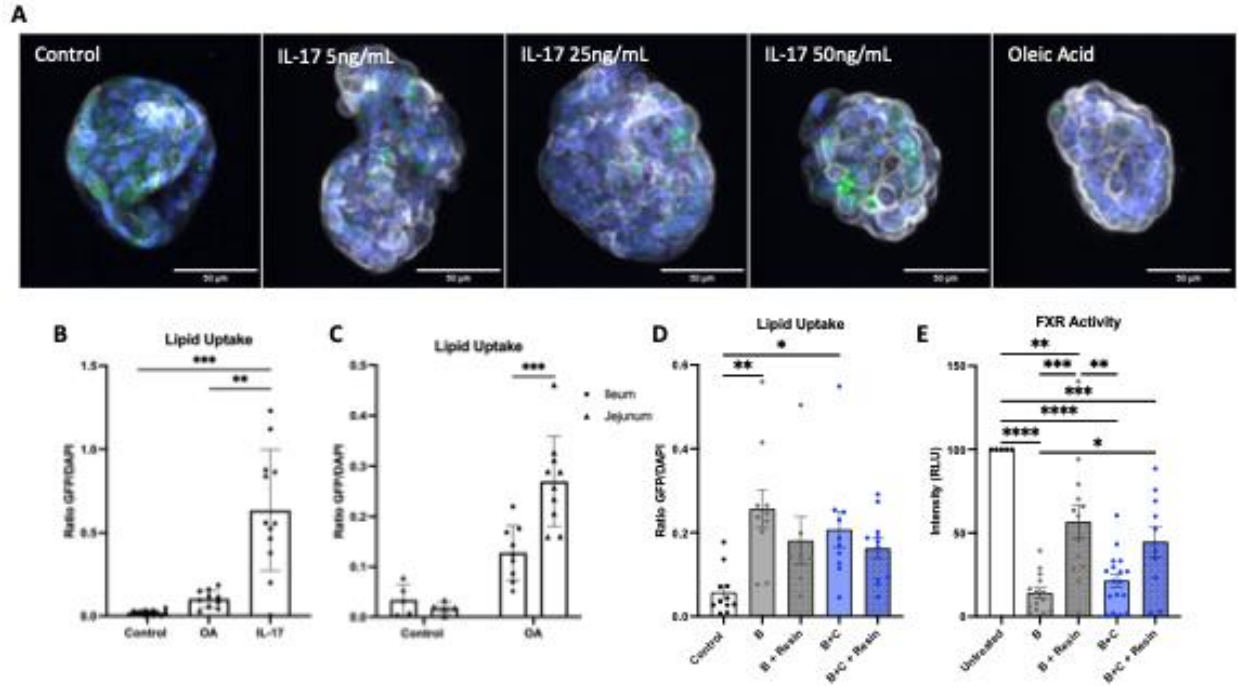


Figure S2. Validation of the enteroid lipid uptake assay and bile acid-dependent effects on intestinal lipid absorption.

A) Representative confocal images of inverted jejunal enteroids exposed to increasing concentrations of IL-17 (0, 5, 25 or 50 ng/mL) in the presence of oleic acid or in its absence (control). Lipid droplets were stained with BODIPY AF488, F-actin with phalloidin AF647, and nuclei with DAPI. **B)** Quantification of jejunal enteroid lipid uptake following IL-17 treatment, expressed as BODIPY (GFP) fluorescence volume normalized to DAPI fluorescence volume. Statistical comparisons performed by one-way ANOVA. Control $n = 13$, oleic acid (OA) $n = 10$, IL-17 $n = 13$. **C)** Comparison of lipid uptake between jejunal and ileal enteroids following oleic acid treatment, expressed as BODIPY (GFP) fluorescence volume normalized to DAPI fluorescence volume. Statistical comparisons performed by two-way ANOVA. Control ileum $n = 5$, control jejunum $n = 5$, OA ileum $n = 9$, OA jejunum $n = 10$. **D)** Quantification of lipid uptake in ileal enteroids exposed to sterile fecal filtrates from B or B+C mice in the presence or absence of cholestyramine resin, expressed as BODIPY (GFP) fluorescence volume normalized to DAPI fluorescence volume. Statistical comparisons performed by one-way ANOVA. Untreated: control $n = 12$, B $n = 10$, B+C $n = 10$; cholestyramine: B $n = 8$, B+C $n = 12$. **E)** Farnesoid X receptor (FXR) reporter activity following exposure to sterile fecal filtrates from B or B+C mice with or without cholestyramine. Statistical comparisons performed by one-way ANOVA. Untreated: control $n = 5$, B $n = 10$, B+C $n = 10$; cholestyramine: B $n = 8$, B+C $n = 12$. Data represented as mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

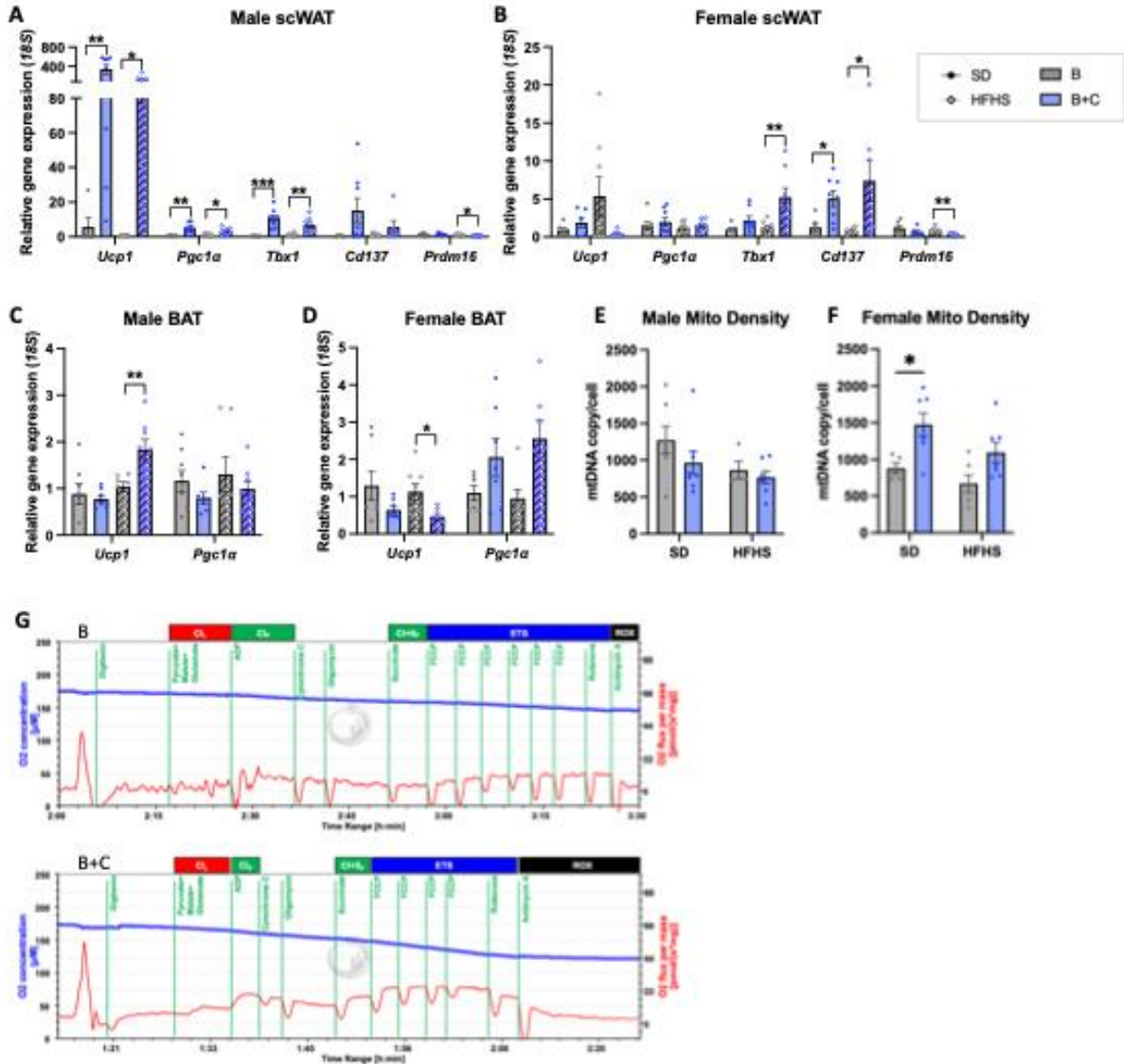
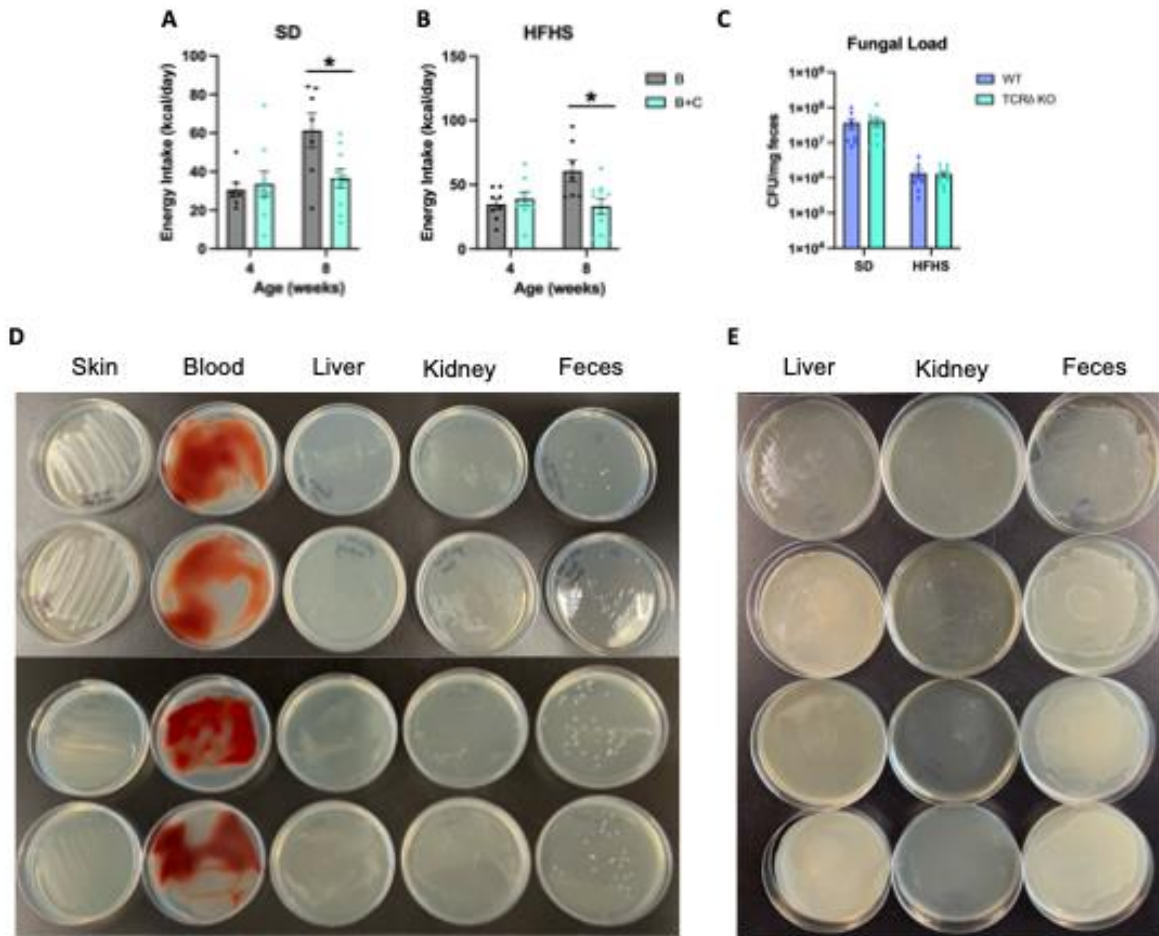


Figure S3. *C. albicans* colonization promotes thermogenesis gene expression in subcutaneous and brown adipose tissue and increases mitochondrial respiration in gWAT.

A–D) Relative expression normalized to *18S* (calculated using $2^{-\Delta\Delta C_t}$) of thermogenesis-associated genes in subcutaneous white adipose tissue (scWAT; **A–B**) and brown adipose tissue (BAT; **C–D**) from male (**A, C**) and female (**B, D**) mice. Outliers were removed using ROUT ($Q = 1\%$). Normality was assessed using the Shapiro–Wilk test, and statistical significance within each diet was determined using an unpaired t -test or Mann–Whitney U test, as appropriate. **E–F)** Mitochondrial DNA (mtDNA) copy number per diploid cell in gWAT from male (**E**) and female (**F**) mice. Statistical significance was determined by two-way ANOVA followed by Šidák's multiple comparisons test. **G)** Representative high-resolution respirometry trace (oxygraph) from gonadal white adipose tissue of B and B+C male mice fed a standard diet. Data represented as mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.



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Figure S4. $\gamma\delta$ cell-deficient mice maintain intestinal *C. albicans* colonization without microbial dissemination.

A–B) Average daily energy intake of $\gamma\delta$ TCR-deficient mice fed a standard diet (SD; **A**) or high-fat high-sucrose diet (HFHS; **B**). Each point represents the mean daily energy intake of an individual cage. Outliers were removed using ROUT ($Q = 1\%$). Normality was assessed using the Shapiro–Wilk test, and comparisons between B and B+C groups at each time point were performed using unpaired t -tests. **C)** Quantification of fecal *C. albicans* colony-forming units (CFUs) in wild-type and $\gamma\delta$ TCR-deficient mice at 10 weeks of age. Statistical significance was determined by two-way ANOVA followed by Šídák's multiple comparisons test. **D)** Representative fungal cultures in yeast-mold agar inoculated with feces, liver, kidney, blood and skin collected from $\gamma\delta$ TCR-deficient B+C mice following 48 h incubation at room temperature. **E)** Representative bacterial cultures in fastidious anaerobe agar inoculated with feces, liver, kidney, blood and skin collected from $\gamma\delta$ TCR-deficient B+C mice following 48 h anaerobic incubation at 37°C. Data represented as mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

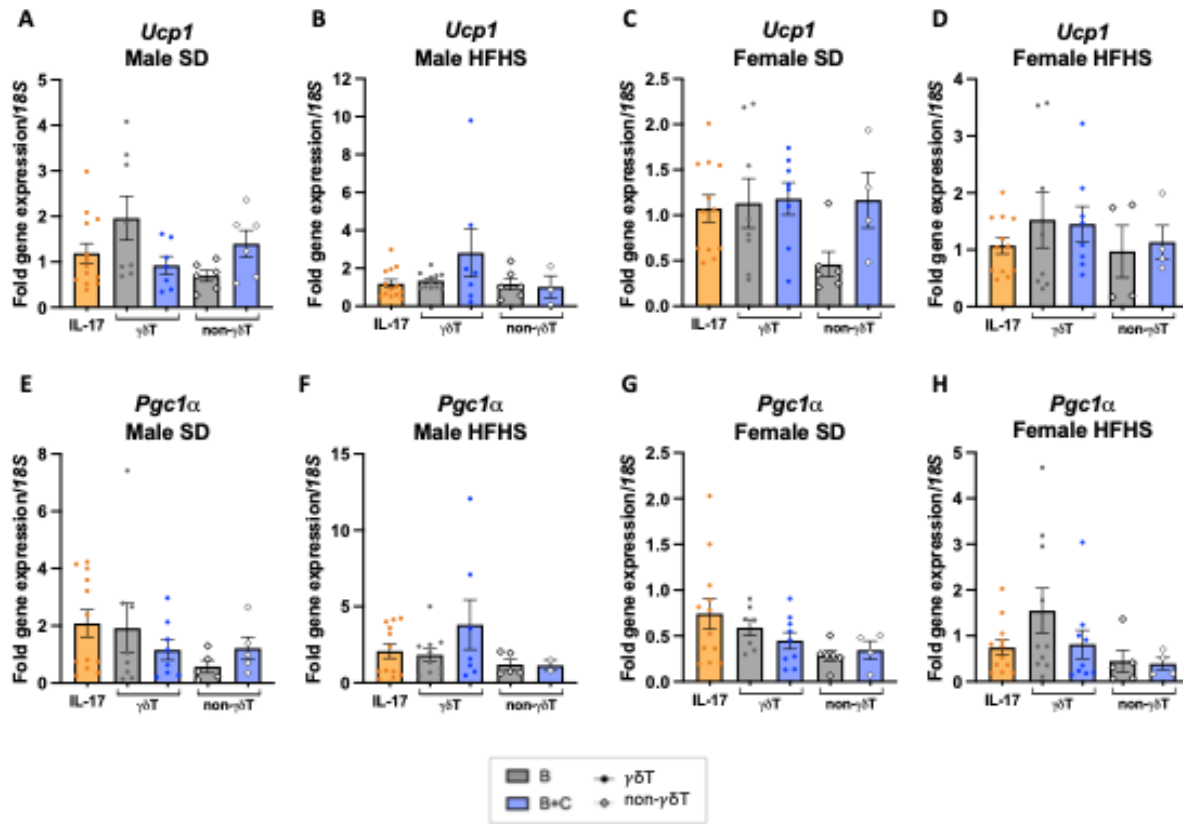


Figure S5: WAT $\gamma\delta$ T cells from *C. albicans* colonized mice are insufficient to induce thermogenic gene expression in primary differentiated adipocytes.

A–D) Relative expression of *Ucp1* in primary differentiated adipocytes co-cultured with $\gamma\delta$ T cells and non- $\gamma\delta$ T cells isolated from gWAT of male and female mice fed either a standard diet (SD) or high-fat high-sucrose diet (HFHS). **E–H)** Relative expression of *Pgc1α* in primary differentiated adipocytes co-cultured with $\gamma\delta$ T cells and non- $\gamma\delta$ T cells isolated from gWAT of male and female mice fed either SD or HFHS. Outliers removed with ROUT ($Q = 1\%$).

Normality assessed by Shapiro-Wilk test and significant differences defined by One-way ANOVA with Tukey's multiple comparisons test or Kruskal-Wallis test with Dunn's multiple comparisons test, accordingly. Data represented as mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

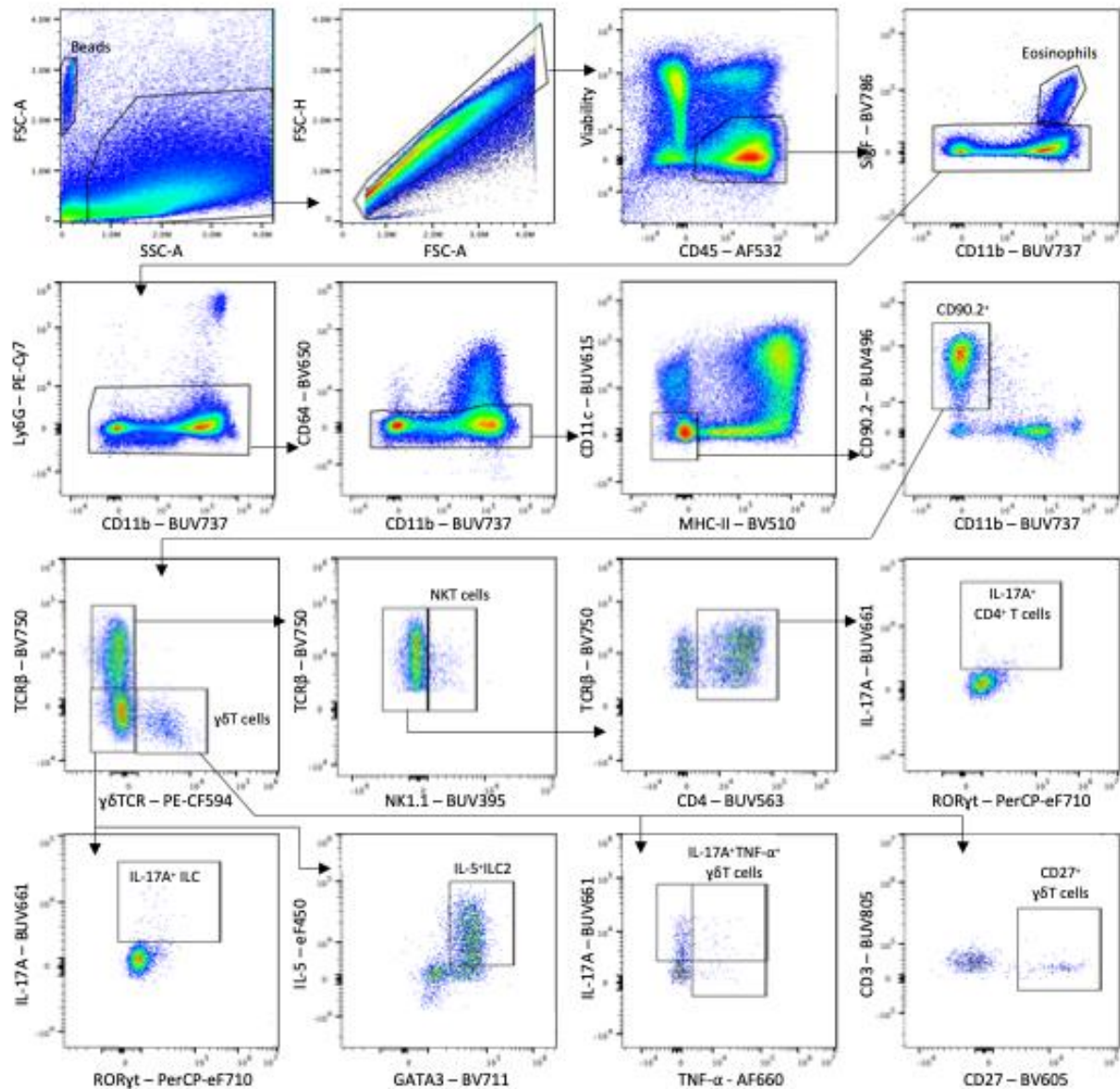


Figure S6. Flow cytometry gating strategy for adipose tissue immune cells.

Representative flow cytometry gating strategy used to identify immune cell populations in gWAT. Sequential gating was used to identify eosinophils, $\gamma\delta$ T cells, IL-17A⁺TNF- α ⁺ $\gamma\delta$ T cells, IL-17A⁺ innate lymphoid cells (ILCs), natural killer T (NKT) cells, IL-17A⁺CD4⁺ T cells, IL-5⁺ ILC2s, and CD27⁺ $\gamma\delta$ T cells.

Bile acid	B mean	B+C mean	Fold change (B+C vs B)	p-value	FDR
Chenodeoxycholic acid (CDCA)	2.928	2.339	0.799	0.251	0.43
Deoxycholic acid (DCA)	0.051	0.217	4.222	0.000358	0.0043
Ursodeoxycholic acid (UCA)	0.242	0.285	1.175	0.185	0.371
Alpha-muricholic acid (MCA)	45.326	57.023	1.258	0.00619	0.0372
Beta-muricholic acid (MCA)	250.362	233.26	0.932	0.93	0.93
Cholic acid (CA)	107.292	91.777	0.855	0.659	0.791
Tauroursodeoxycholic acid (TUDCA)	1.168	1.023	0.876	0.289	0.434
Taurohyodeoxycholic acid (THDCA)	17.124	11.751	0.686	0.0934	0.336
Taurochenodeoxycholic acid (TCDCA)	1.417	0.966	0.681	0.112	0.336
Taurodeoxycholic acid (TDCA)	9.847	6.622	0.672	0.158	0.371
Tauro-beta-muricholic acid (T-beta MCA)	158.442	144.345	0.911	0.331	0.442
Taurocholic acid (TCA)	54.177	51.171	0.945	0.93	0.93

Table S1. Fecal bile acids. Statistics from Mann–Whitney U test with FDR correction.

Metric	B mean	B+C mean	Fold change (B+C vs B)	p-value	FDR	Contributing bile acids
Primary unconjugated	405.908	384.399	0.947	0.93	1	CDCA, alpha-MCA, beta-MCA, CA
Primary conjugated	214.037	196.481	0.918	0.185	0.525	TCDCA, T-beta-MCA, TCA
Secondary unconjugated	0.294	0.502	1.709	0.00268	0.0152	DCA, UCA
Secondary conjugated	28.139	19.395	0.689	0.00268	0.0152	TUDCA, THDCA, TDCA
Total primary	619.945	580.881	0.937	0.93	1	CDCA, alpha-MCA, beta-MCA, CA, TCDCA, T-beta-MCA, TCA
Total secondary	28.433	19.897	0.7	0.00268	0.0152	DCA, UCA, TUDCA, THDCA, TDCA
Total conjugated	242.176	215.876	0.891	0.112	0.381	TCDCA, T-beta-MCA, TCA, TUDCA, THDCA, TDCA

Total unconjugated	406.202	384.902	0.948	0.93	1	CDCA, alpha-MCA, beta-MCA, CA, DCA, UCA
Pool 12a OH	171.368	149.787	0.874	0.536	0.829	CA, TCA, DCA, TDCA
Pool non 12a	477.01	450.991	0.945	1	1	CDCA, alpha-MCA, beta-MCA, UCA, TCDCA, T-beta-MCA, TUDCA, THDCA
Muricholate pool	454.131	434.628	0.957	1	1	alpha-MCA, beta-MCA, T-beta-MCA
Hydrophobic pool	175.713	153.092	0.871	0.536	0.829	CA, TCA, CDCA, TCDCA, DCA, TDCA
Hydrophilic pool	472.665	447.686	0.947	1	1	alpha-MCA, beta-MCA, T-beta-MCA, UCA, TUDCA, THDCA
Secondary:primary	0.05	0.035	0.703	0.0423	0.18	Total_Secondary / Total_Primary
Conjugated:total	0.392	0.354	0.903	0.427	0.806	Total_Conjugated / (Total_Conjugated + Total_Unconjugated)
12a:non12a	0.341	0.323	0.947	0.251	0.533	Pool_12a_OH / Pool_non_12a
Hydrophobic:hydrophilic	0.354	0.332	0.939	0.251	0.533	Hydrophobic_Pool / Hydrophilic_Pool

Table S2. Fecal bile acid categories. Statistics from Mann–Whitney U test with FDR correction.

Population	B	B+C	Mean diff± SEM	95% CI	p-value
Eosinophils	5.477	3.614	-1.863 ± 0.4963	-2.921 to -0.8051	0.0019
$\gamma\delta$ T cells	9.441	23.19	13.75 ± 1.538	10.47 to 17.02	<0.0001
CD27+ $\gamma\delta$ T cells	14.60	6.358	-8.246 ± 2.161	-12.85 to -3.64	0.0017
TNF+IL1 $\gamma\delta$ T cells	5.431	15.11	9.683 ± 3.877	1.367 to 18.00	0.0256
NKT cells	6.941	6.208	-0.7336 ± 1.007	-2.880 to 1.413	0.4775
IL17+ CD4+ T cells	2.101	4.776	2.675 ± 0.4555	1.698 to 3.652	<0.0001
IL17+ ILC	1.948	4.904	2.956 ± 0.8163	1.206 to 4.707	0.0028
IL5+GATA3+ ILC	58.84	40.10	-18.74 ± 2.894	-24.91 to -12.58	<0.0001

Table S3. WAT thermogenesis-associated immune population summary statistics in males fed SD. Statistics from unpaired T-test (B n=9, B+C n=8).

Population	B	B+C	Mean diff± SEM	95% CI	p-value
Eosinophils	4.728	1.359	-3.369 ± 0.7109	-4.876 to -1.862	0.0002
$\gamma\delta$ T cells	9.368	20.76	11.39 ± 1.332	8.570 to 14.22	<0.0001
CD27+ $\gamma\delta$ T cells	18.39	10.95	-7.435 ± 1.154	-9.894 to -4.976	<0.0001
TNF+IL17+ $\gamma\delta$ T cells	4.426	16.33	11.90 ± 1.013	9.743 to 14.06	<0.0001
NKT cells	4.767	9.334	4.567 ± 1.969	0.3936 to 8.740	0.0339

IL17+ CD4+ T cells	2.182	4.365	2.183 ± 0.6035	0.9036 to 3.462	0.0023
IL17+ ILC	2.863	6.364	3.501 ± 1.027	1.323 to 5.679	0.0036
IL5+GATA3+ ILC	44.59	31.30	-13.29 ± 3.822	-21.39 to -5.188	0.0031

Table S4. WAT thermogenesis-associated immune population summary statistics in males fed HFHS. Statistics from unpaired T-test (B n=10, B+C n=8).

Population	B	B+C	Mean diff± SEM	95% CI	p-value
Eosinophils	10.51	11.13	0.6214 ± 1.491	-2.500 to 3.743	0.6816
$\gamma\delta$ Tcells	4.475	10.12	5.642 ± 0.8335	3.897 to 7.386	<0.0001
CD27+ gdT cells	38.50	23.60	-14.90 ± 3.886	-23.03 to -6.766	0.0011
TNF+IL17+ $\gamma\delta$ T cells	4.927	19.62	14.69 ± 2.658	9.127 to 20.25	<0.0001
NKT cells	5.348	6.782	1.435 ± 1.522	-1.750 to 4.620	0.3576
IL17+ CD4+ T cells	1.985	4.478	2.492 ± 0.5349	1.369 to 3.616	0.0002
IL17+ ILC	1.503	7.222	5.719 ± 0.6075	4.443 to 6.996	<0.0001
IL5+GATA3+ ILC	61.20	56.07	-5.133 ± 4.043	-13.59 to 3.328	0.2195

Table S5. WAT thermogenesis-associated immune population summary statistics in females fed SD. Statistics from unpaired T-test (B n=12, B+C n=9).

Population	B	B+C	Mean diff± SEM	95% CI	p-value
Eosinophils	8.164	3.844	-4.319 ± 0.9173	-6.246 to -2.392	0.0002
$\gamma\delta$ Tcells	6.099	12.31	6.214 ± 0.9788	4.158 to 8.271	<0.0001
CD27+ $\gamma\delta$ T cells	44.75	24.63	-20.12 ± 3.967	-28.46 to -11.79	<0.0001
TNF+IL17+ $\gamma\delta$ T cells	5.901	14.92	9.024 ± 3.338	2.011 to 16.04	0.0145
NKT cells	5.009	8.631	3.622 ± 1.064	1.386 to 5.858	0.0032
IL17+ CD4+ T cells	1.878	5.143	3.265 ± 0.6549	1.889 to 4.641	<0.0001
IL17+ ILC	3.305	7.340	4.035 ± 1.443	1.003 to 7.066	0.0119
IL5+GATA3+ ILC	51.55	42.50	-9.045 ± 5.730	-21.08 to 2.992	0.1318

Table S6. WAT thermogenesis-associated immune population summary statistics in females fed HFHS. Statistics from unpaired T-test (B n=11, B+C n=9).