

# Supplementary Materials for

## Early-life gut mycobiome core species modulate metabolic health in mice

Mackenzie W. Gutierrez *et al.*

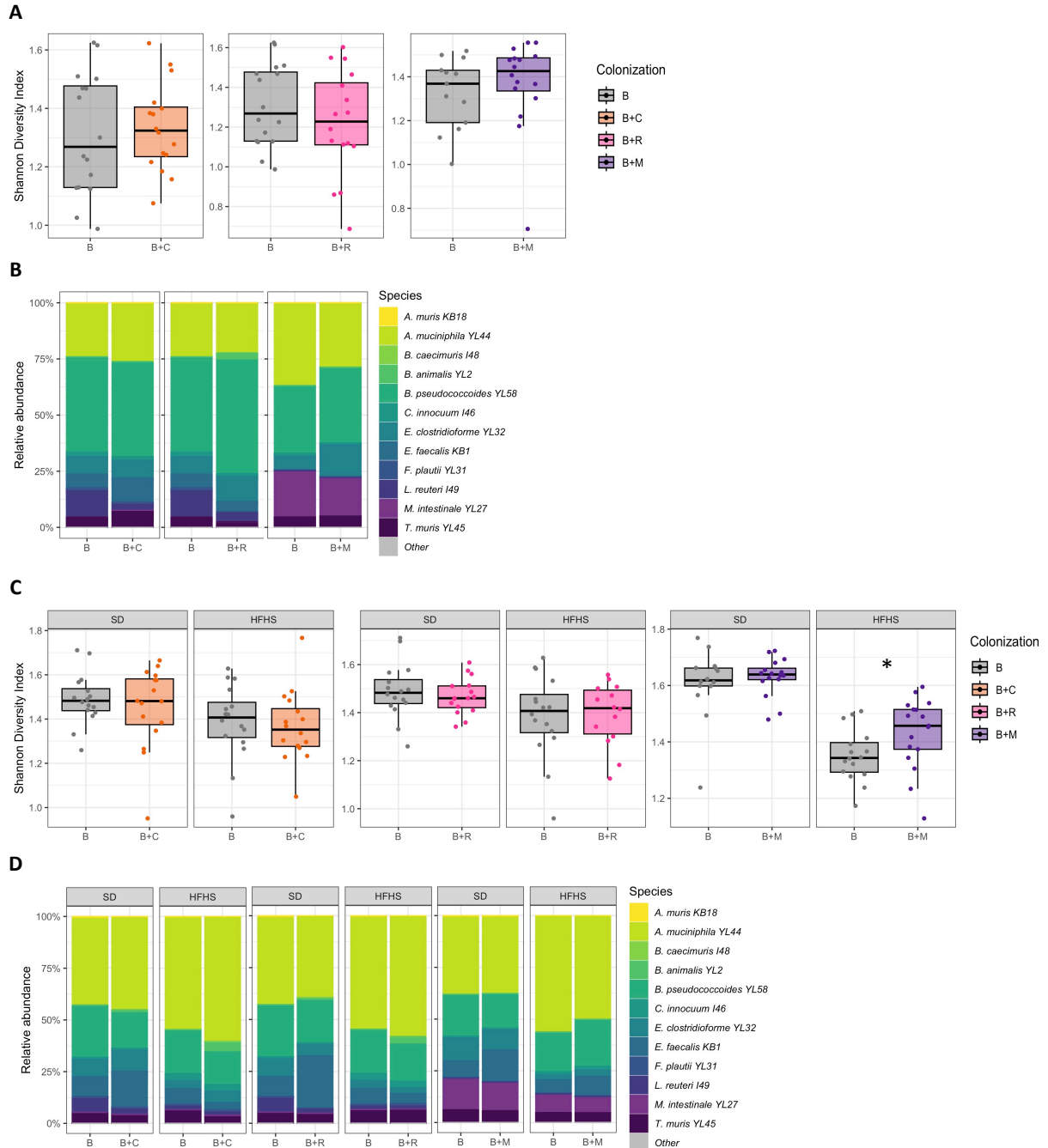
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### **This PDF file includes:**

Figs. S1 to S8  
Tables S1 to S13

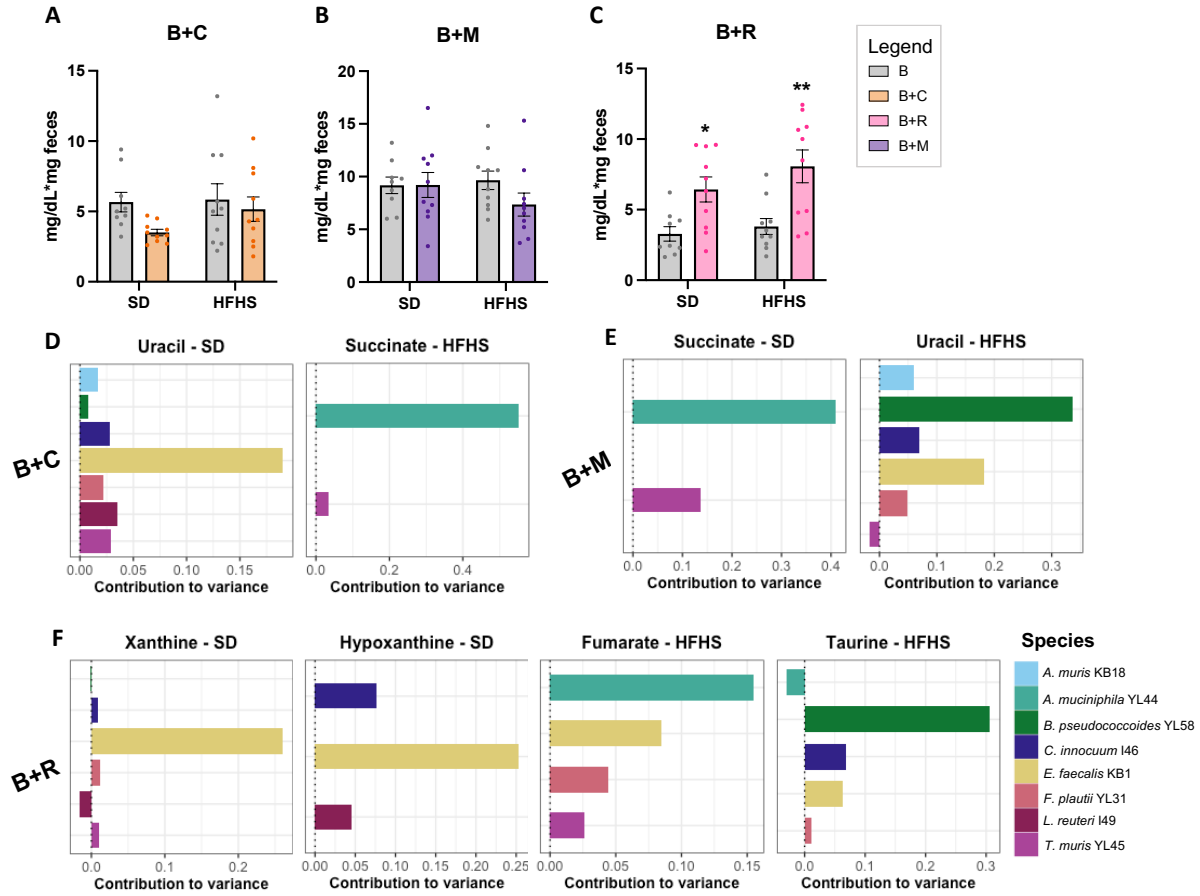
### **Other Supplementary Materials for this manuscript include the following:**

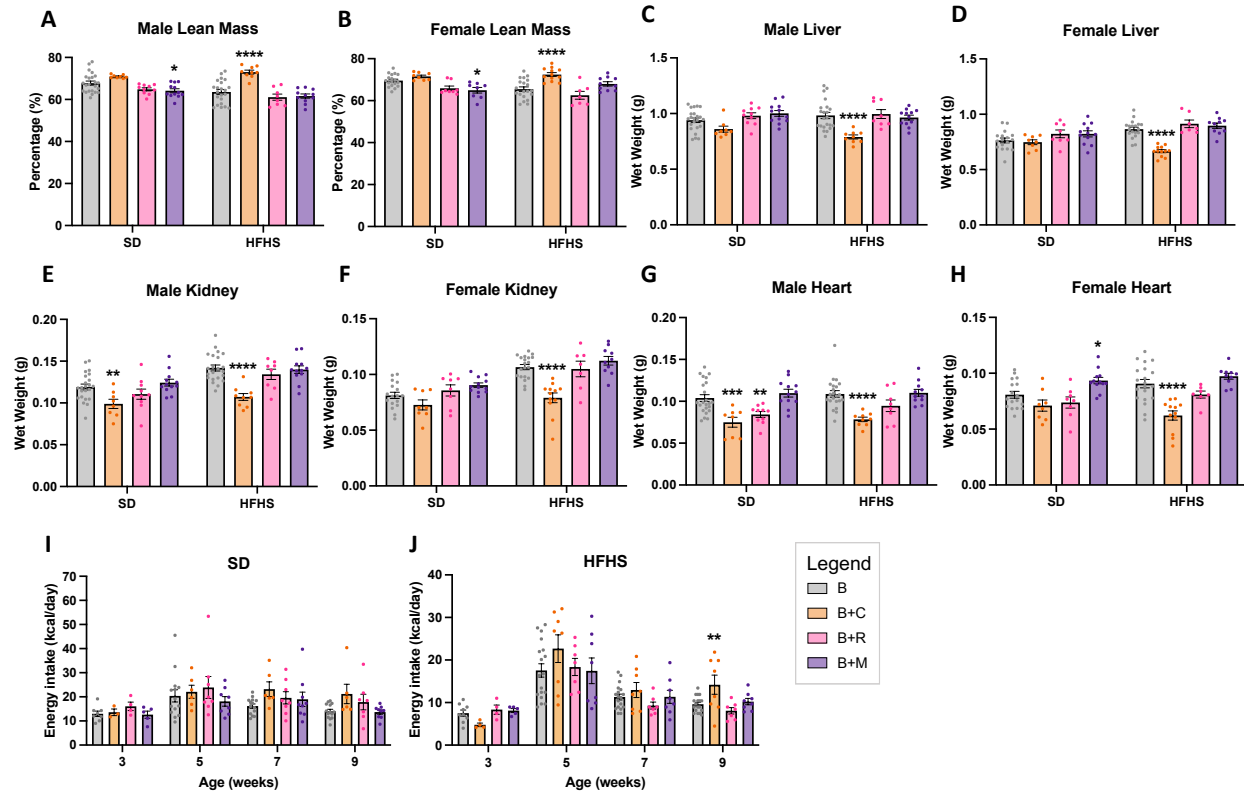
Source Data  
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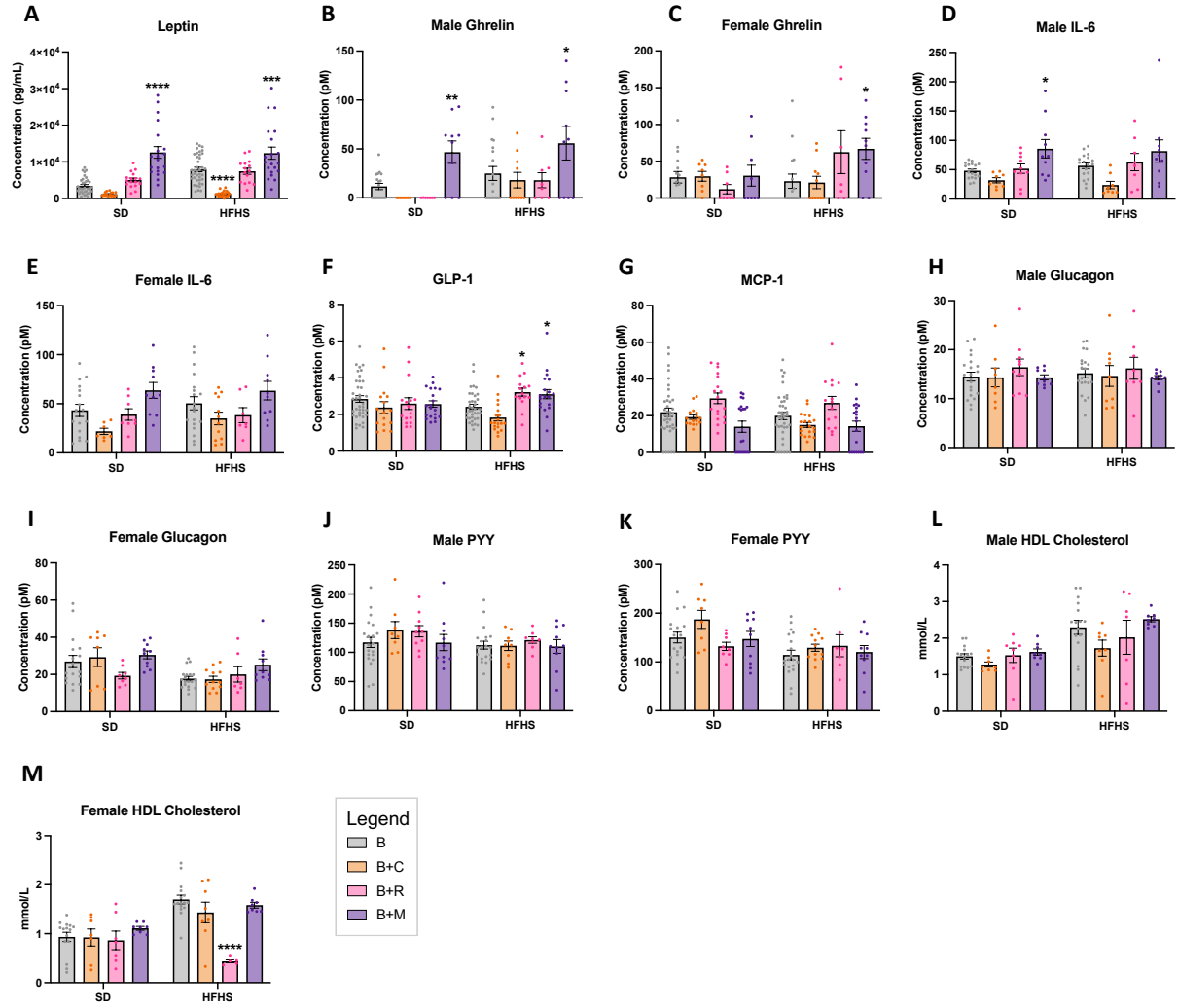
**Figure S1. Bacterial Shannon diversity and relative abundance with fungal colonization (related to Figure 2).** (A) Bacterial Shannon diversity index at 3 weeks of age between B only and B+C ( $n_B = 16$ ,  $n_{B+C} = 16$ ), B+R ( $n_B = 16$ ,  $n_{B+R} = 16$ ) and B+M ( $n_B = 13$ ,  $n_{B+M} = 16$ ). (B) Bacterial relative abundance at 3 weeks of age between B only and B+C ( $n_B = 16$ ,  $n_{B+C} = 16$ ), B+R ( $n_B = 16$ ,  $n_{B+R} = 16$ ) and B+M ( $n_B = 13$ ,  $n_{B+M} = 16$ ). (C) Bacterial Shannon diversity at 12 weeks of age by diet between B only and B+C (SD:  $n_B = 16$ ,  $n_{B+C} = 16$ ; HFHS:  $n_B = 16$ ,  $n_{B+C} = 16$ ), B+R (SD:  $n_B = 16$ ,  $n_{B+R} = 16$ ; HFHS:  $n_B = 16$ ,  $n_{B+R} = 14$ ) and B+M (SD:  $n_B = 14$ ,  $n_{B+M} = 16$ ; HFHS:  $n_B = 16$ ,  $n_{B+M} = 17$ ). (D) Bacterial relative abundance at 12 weeks of age by diet between B only and B+C (SD:  $n_B = 16$ ,  $n_{B+C} = 16$ ; HFHS:  $n_B = 16$ ,  $n_{B+C} = 16$ ), B+R (SD:  $n_B = 16$ ,  $n_{B+R} =$

16; HFHS:  $n_B = 16$ ,  $n_{B+R} = 14$ ) and B+M (SD:  $n_B = 14$ ,  $n_{B+M} = 16$ ; HFHS:  $n_B = 16$ ,  $n_{B+M} = 17$ ). Boxplots show median and interquartile range with whiskers representing the largest or smallest values no further than 1.5 times the IQR from the hinge. Normality assessed with the Shapiro-Wilk test and differences calculated with unpaired T-test or Mann-Whitney test accordingly. (A & C) B = grey; B+C = orange; B+R = pink; B+M = purple. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . Source data are provided as a source data file; n indicates number of mice sampled. SD, standard diet; HFHS, high-fat-high-sucrose diet.



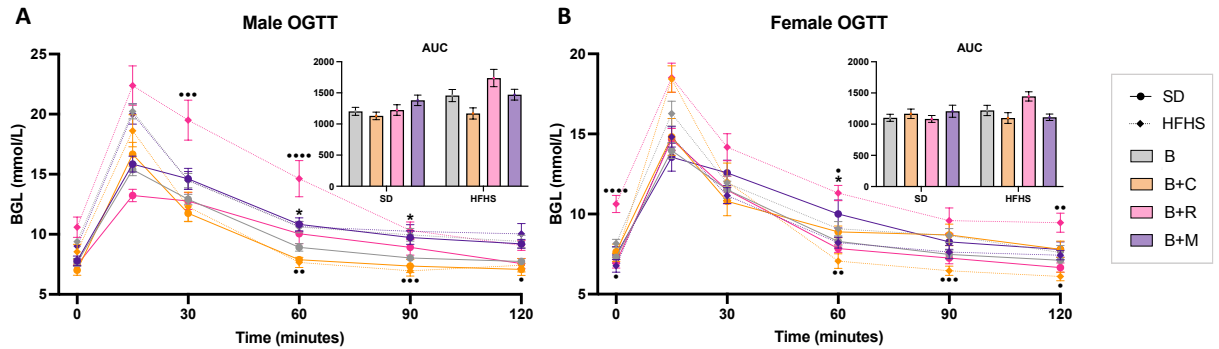


provided as a source data file; n indicates number of mice sampled. SD, standard diet; HFHS, high-fat-high-sucrose diet.

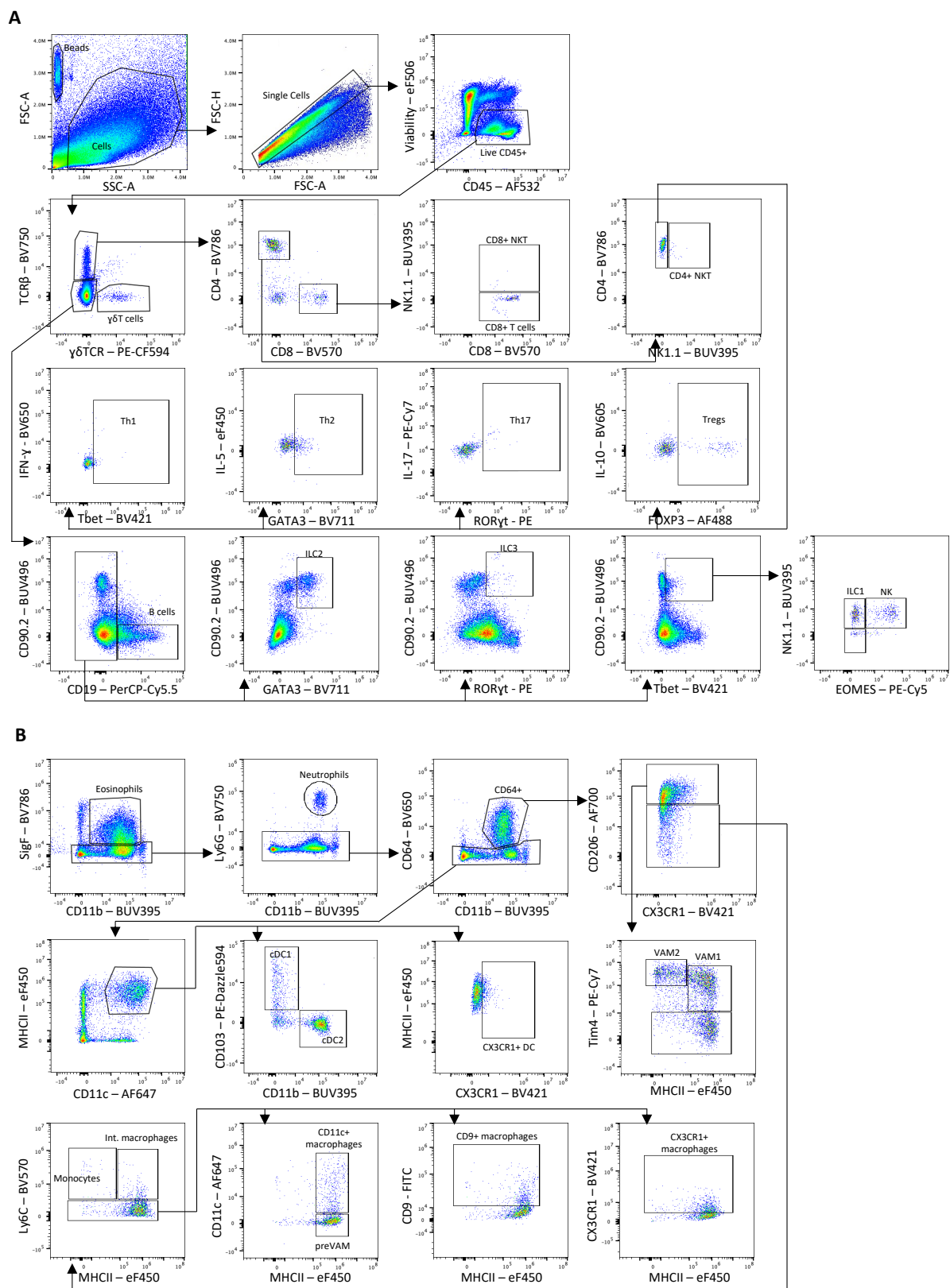


**Figure S4. Fungal colonization modulates metabolic hormones and cytokines in plasma (related to Figure 6).** (A) Plasma leptin independent of sex (SD:  $n_B = 38$ ,  $n_{B+C} = 16$ ,  $n_{B+R} = 18$ ,  $n_{B+M} = 20$ ; HFHS:  $n_B = 39$ ,  $n_{B+C} = 21$ ,  $n_{B+R} = 15$ ,  $n_{B+M} = 20$ ). (B-C) Plasma ghrelin in males and females, respectively (Male – SD:  $n_B = 21$ ,  $n_{B+C} = 8$ ,  $n_{B+R} = 10$ ,  $n_{B+M} = 10$ ; Male – HFHS:  $n_B = 20$ ,  $n_{B+C} = 9$ ,  $n_{B+R} = 8$ ,  $n_{B+M} = 10$ ; Female – SD:  $n_B = 17$ ,  $n_{B+C} = 8$ ,  $n_{B+R} = 8$ ,  $n_{B+M} = 10$ ; Female – HFHS:  $n_B = 18$ ,  $n_{B+C} = 12$ ,  $n_{B+R} = 7$ ,  $n_{B+M} = 10$ ). (D-E) Plasma IL-6 in males and females, respectively (Male – SD:  $n_B = 21$ ,  $n_{B+C} = 8$ ,  $n_{B+R} = 10$ ,  $n_{B+M} = 10$ ; Male – HFHS:  $n_B = 20$ ,  $n_{B+C} = 9$ ,  $n_{B+R} = 8$ ,  $n_{B+M} = 10$ ; Female – SD:  $n_B = 17$ ,  $n_{B+C} = 8$ ,  $n_{B+R} = 8$ ,  $n_{B+M} = 10$ ; Female – HFHS:  $n_B = 19$ ,  $n_{B+C} = 12$ ,  $n_{B+R} = 7$ ,  $n_{B+M} = 10$ ). (F) Plasma GLP-1 independent of sex (SD:  $n_B = 38$ ,  $n_{B+C} = 16$ ,  $n_{B+R} = 18$ ,  $n_{B+M} = 20$ ; HFHS:  $n_B = 39$ ,  $n_{B+C} = 21$ ,  $n_{B+R} = 15$ ,  $n_{B+M} = 20$ ). (G) Plasma MCP-1 independent of sex (SD:  $n_B = 38$ ,  $n_{B+C} = 16$ ,  $n_{B+R} = 18$ ,  $n_{B+M} = 20$ ; HFHS:  $n_B = 39$ ,  $n_{B+C} = 21$ ,  $n_{B+R} = 15$ ,  $n_{B+M} = 20$ ). (H-I) Plasma glucagon in males and females, respectively (Male – SD:  $n_B = 21$ ,  $n_{B+C} = 8$ ,  $n_{B+R} = 10$ ,  $n_{B+M} = 10$ ; Male – HFHS:  $n_B = 20$ ,  $n_{B+C} = 9$ ,  $n_{B+R} = 8$ ,  $n_{B+M} = 10$ ; Female – SD:  $n_B = 17$ ,  $n_{B+C} = 8$ ,  $n_{B+R} = 8$ ,  $n_{B+M} = 10$ ; Female – HFHS:  $n_B = 19$ ,  $n_{B+C} = 12$ ,  $n_{B+R} = 7$ ,  $n_{B+M} = 10$ ). (J-K) Plasma PYY in males and females, respectively (Male – SD:  $n_B = 21$ ,  $n_{B+C} = 8$ ,  $n_{B+R} = 10$ ,  $n_{B+M} = 10$ ; Male – HFHS:  $n_B = 20$ ,  $n_{B+C} = 9$ ,  $n_{B+R} = 8$ ,  $n_{B+M} = 10$ ; Female – SD:  $n_B = 17$ ,  $n_{B+C} = 8$ ,  $n_{B+R} = 8$ ,  $n_{B+M} = 10$ ; Female – HFHS:  $n_B = 19$ ,  $n_{B+C} = 12$ ,  $n_{B+R} = 7$ ,  $n_{B+M} = 10$ ). (L-M) Plasma HDL cholesterol in males and females, respectively (Male – SD:  $n_B = 15$ ,  $n_{B+C} =$

8,  $n_{B+R} = 8$ ,  $n_{B+M} = 8$ ; Male – HFHS:  $n_B = 16$ ,  $n_{B+C} = 8$ ,  $n_{B+R} = 7$ ,  $n_{B+M} = 8$ ; Female – SD:  $n_B = 15$ ,  $n_{B+C} = 7$ ,  $n_{B+R} = 7$ ,  $n_{B+M} = 8$ ; Female – HFHS:  $n_B = 16$ ,  $n_{B+C} = 8$ ,  $n_{B+R} = 7$ ,  $n_{B+M} = 8$ ). Data represents mean  $\pm$  SEM. Outliers removed with ROUT (Q = 1%) and significant differences at each timepoint defined by Two-way ANOVA with Dunnett's multiple comparisons test relative to B only group within either diet. B = grey; B+C = orange; B+R = pink; B+M = purple. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ . Source data are provided as a source data file; n indicates number of mice sampled. SD, standard diet; HFHS, high-fat-high-sucrose diet; HDL, high-density lipoprotein.

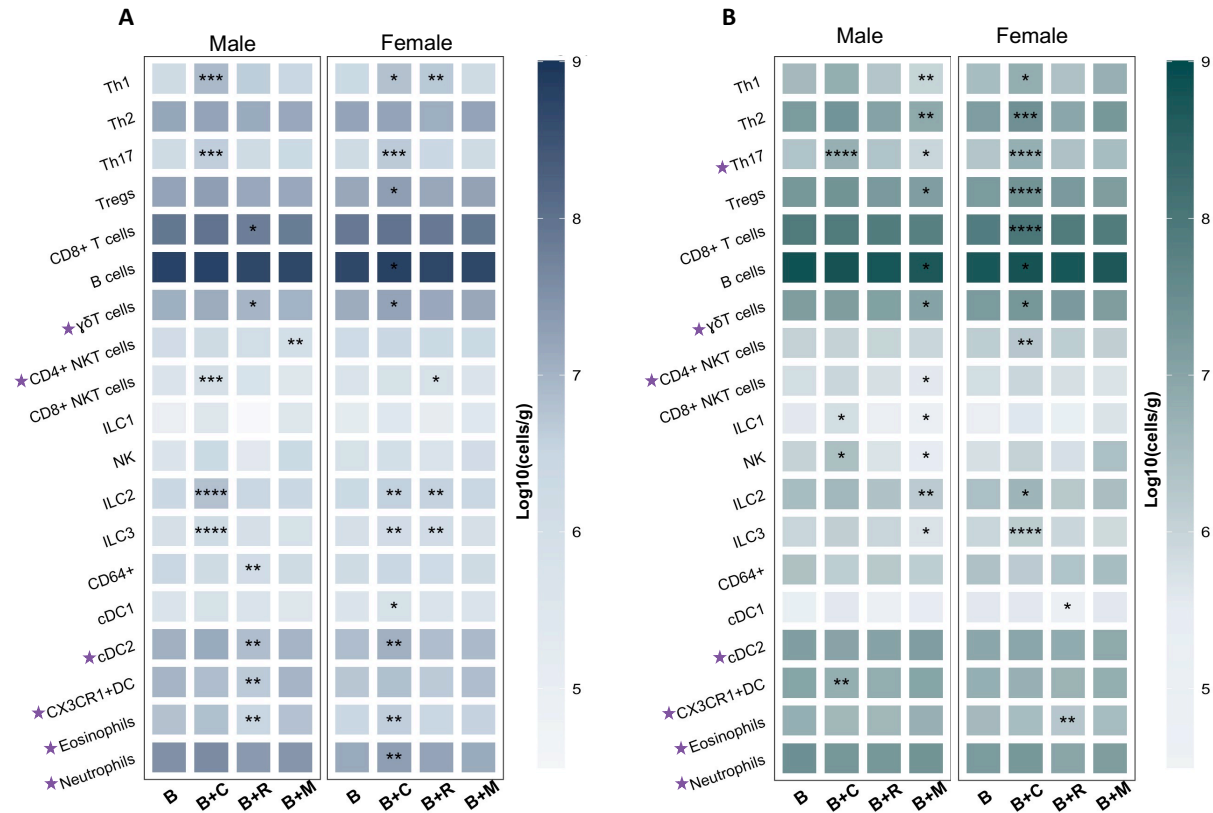


**Figure S5. Fecal filtrates do not modulate nutrient transporter expression in jejunal enteroids.** (A) Expression of *Cd36* in enteroids treated with fecal filtrates obtained from mice colonized with bacteria alone (B) or in combination with each of the three fungal species studied ( $n_B = 4$ ,  $n_{B+C} = 4$ ,  $n_{B+R} = 4$ ,  $n_{B+M} = 4$ ,  $n_{B-IL17} = 3$ ,  $n_{B+C-IL17} = 3$ ,  $n_{B+R-IL17} = 4$ ,  $n_{B+M-IL17} = 4$ ). (B) Expression of *Slc5a1* in enteroids treated with fecal filtrates (B  $n = 2$ ,  $n_{B+C} = 3$ ,  $n_{B+R} = 3$ ,  $n_{B+M} = 2$ ,  $n_{B-IL17} = 3$ ,  $n_{B+C-IL17} = 3$ ,  $n_{B+R-IL17} = 4$ ,  $n_{B+M-IL17} = 3$ ). Data mean  $\pm$  SEM. Outliers removed with ROUT (Q = 1%) and significant differences at each timepoint defined by Two-way ANOVA with Dunnett's multiple comparisons test relative to B only group within either treatment. B = grey; B+C = orange; B+R = pink; B+M = purple. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ . Source data are provided as a source data file; n indicates number of experiments.

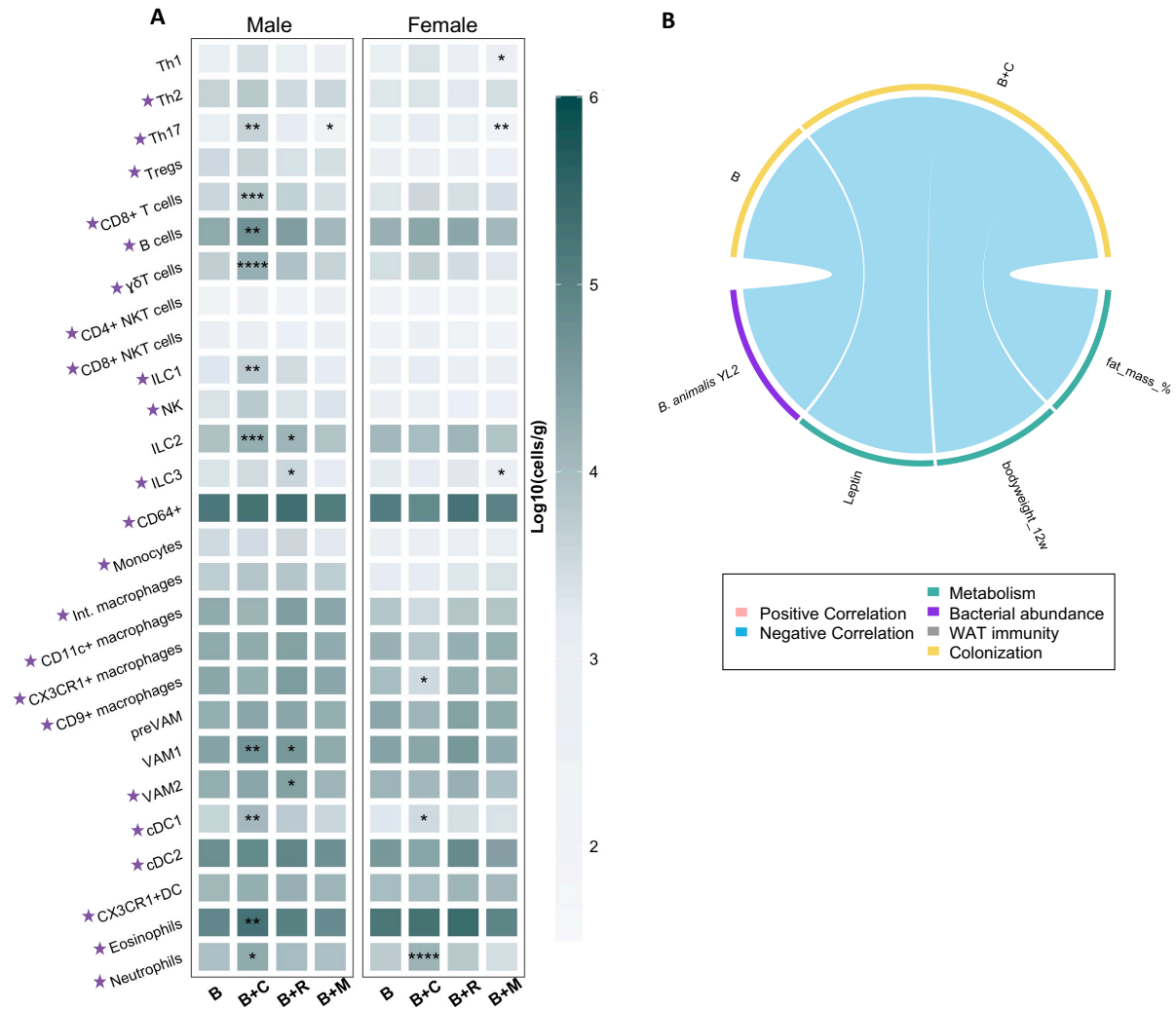


**Figure S6. Flow cytometry gating strategy (related to Figure 7). (A) Lymphoid panel gating strategy. (B) Myeloid panel gating strategy following selection of Live CD45+ cells as shown in**

A. Data shown is from WAT immune cells. Gating corresponds to Figures 7, S7 & S8. Source data are provided as a source data file.



**Figure S7. Fungal colonization modulates spleen immune cell populations (related to Figure 7).** (A-B) Spleen immune cell populations mice fed SD (Male:  $n_B = 23$ ,  $n_{B+C} = 8$ ,  $n_{B+R} = 10$ ,  $n_{B+M} = 10$ ; Female:  $n_B = 17$ ,  $n_{B+C} = 8$ ,  $n_{B+R} = 8$ ,  $n_{B+M} = 11$ ) and HFHS (Male:  $n_B = 22$ ,  $n_{B+C} = 9$ ,  $n_{B+R} = 8$ ,  $n_{B+M} = 6$ ; Female:  $n_B = 20$ ,  $n_{B+C} = 12$ ,  $n_{B+R} = 6$ ,  $n_{B+M} = 3$ ), respectively. Data represented as mean log10(cells/gram of tissue). Outliers identified with ROUT ( $Q = 1\%$ ). Sex effect assessed by Three-way ANOVA and  $p < 0.05$  indicated with a star. Significant differences between fungal colonized and B only group defined by Two-way ANOVA with Dunnett's multiple comparisons test. Source data are provided as a source data file; n indicates number of mice sampled. SD, standard diet; HFHS, high-fat-high-sucrose diet.



**Figure S8. Fungi modulate the visceral WAT immune cell landscape, microbiome and metabolism in an integrated manner on HFHS (related to Figure 7).** (A) WAT immune cell populations in HFHS-fed mice (Male:  $n_B = 21$ ,  $n_{B+C} = 9$ ,  $n_{B+R} = 8$ ,  $n_{B+M} = 12$ ; Female:  $n_B = 20$ ,  $n_{B+C} = 10$ ,  $n_{B+R} = 7$ ,  $n_{B+M} = 10$ ). Data represented as mean  $\log_{10}(\text{cells/gram of tissue})$ . Outliers identified with ROUT ( $Q = 1\%$ ). Sex effect assessed by Three-way ANOVA and  $p < 0.05$  indicated with a star. Significant differences between fungal colonized and B only group defined by Two-way ANOVA with Dunnett's multiple comparisons. (B) Spearman correlation in HFHS-fed mice between variables grouped by metabolism, bacterial abundance, white adipose tissue (WAT) immunity and colonization (B  $n = 24$ ,  $n_{B+C} = 11$ ,  $n_{B+R} = 12$ ,  $n_{B+M} = 11$ ). Correlations with coefficients  $> |0.6|$  shown. Positive correlation = pink; negative correlation = blue, metabolism variables = green; bacterial abundance variables = purple; WAT immunity variables = grey; colonization variables = yellow. Source data are provided as a source data file; n indicates number of mice sampled. SD, standard diet; HFHS, high-fat-high-sucrose diet; WAT, white adipose tissue.

| <b>Group</b> | <b>Variable</b> | <b>F.model</b> | <b>R<sup>2</sup></b> | <b>p-value</b> |
|--------------|-----------------|----------------|----------------------|----------------|
| B+C          | Fungi           | 4.0282         | 0.12020              | 0.010          |
|              | Sex             | 0.4836         | 0.01443              | 0.760          |
| B+R          | Fungi           | 7.1792         | 0.19581              | 0.001          |
|              | Sex             | 0.4859         | 0.01325              | 0.077          |
| B+M          | Fungi           | 1.0274         | 0.03701              | 0.393          |
|              | Sex             | 0.7309         | 0.02633              | 0.567          |

**Table S1. Bacterial beta-diversity statistics at three weeks.** Statistical results defined by PERMANOVA for Bray-Curtis dissimilarity.

| <b>Group</b> | <b>Variable</b> | <b>F.model</b> | <b>R<sup>2</sup></b> | <b>p-value</b> |
|--------------|-----------------|----------------|----------------------|----------------|
| B+C          | Fungi           | 18.398         | 0.12705              | 0.001          |
|              | Sex             | 63.688         | 0.43978              | 0.001          |
|              | Diet            | 2.731          | 0.01886              | 0.039          |
| B+R          | Fungi           | 47.371         | 0.27078              | 0.001          |
|              | Sex             | 67.036         | 0.38319              | 0.001          |
|              | Diet            | 2.535          | 0.01449              | 0.083          |
| B+M          | Fungi           | 4.003          | 0.02677              | 0.016          |
|              | Sex             | 84.133         | 0.56264              | 0.001          |
|              | Diet            | 2.397          | 0.01603              | 0.079          |

**Table S2. Bacterial beta-diversity statistics at twelve weeks.** Statistical results defined by PERMANOVA for Bray-Curtis dissimilarity.

| <b>Group</b> | <b>Variable</b> | <b>F.model</b> | <b>R<sup>2</sup></b> | <b>p-value</b> |
|--------------|-----------------|----------------|----------------------|----------------|
| B+C          | Fungi           | 10.610         | 0.09666              | 0.001          |
|              | Diet            | 38.161         | 0.34764              | 0.001          |
| B+R          | Fungi           | 5.7794         | 0.05622              | 0.001          |
|              | Diet            | 37.0133        | 0.36008              | 0.001          |
| B+M          | Fungi           | 1.3327         | 0.01418              | 0.208          |
|              | Diet            | 47.6212        | 0.50686              | 0.001          |

**Table S3. Fecal metabolome PCA statistics.** Statistical results defined by PERMANOVA for Euclidean distance.

| Colonization | Diet | Metabolite                   | Fold change | Fold change p-value (FDR) | Normalized concentration p-value (FDR) |
|--------------|------|------------------------------|-------------|---------------------------|----------------------------------------|
| B+C          | SD   | L-CYSTATHIONINE              | 44.977      | 1.43E-07                  | <0.000001                              |
|              |      | HYPOTAURINE                  | 41.588      | 1.43E-07                  | <0.000001                              |
|              |      | SUCCINATE                    | 3.8693      | 3.82E-07                  | <0.000001                              |
|              |      | FUMARATE                     | 3.2729      | 5.01E-07                  | <0.000001                              |
|              |      | L-ARABITOL                   | 2.3353      | 6.87E-07                  | <0.000001                              |
|              |      | L-GLUTAMINE                  | 2.2406      | 9.06E-07                  | <0.000001                              |
|              |      | OMEGA-HYDROXYDODECANOIC ACID | 5.8958      | 2.74E-06                  | <0.000001                              |
|              |      | ADIPIC ACID                  | 12.49       | 3.79E-05                  | 0.000005                               |
|              |      | 5-HYDROXYINDOLEACETATE       | 0.38238     | 2.38E-05                  | 0.000003                               |
|              |      | 4-METHYL-2-OXOPENTANOIC ACID | 0.45305     | 4.24E-05                  | 0.000007                               |
|              |      | URATE                        | 0.40761     | 0.00024852                | 0.000046                               |
|              |      | 3-HYDROXYBUTANOIC ACID       | 0.33473     | 0.00050758                | 0.000106                               |
|              |      | FERULATE                     | 0.37692     | 0.0010363                 | 0.000229                               |
|              |      | 6-CARBOXYHEXANOATE           | 0.45197     | 0.01671                   | 0.006606                               |
| B+C          | HFHS | L-ARABITOL                   | 4.4372      | 2.86E-07                  | <0.000001                              |
|              |      | OMEGA-HYDROXYDODECANOIC ACID | 4.2689      | 0.00032946                | 0.000009                               |
|              |      | ADENINE                      | 3.4621      | 0.00079865                | 0.000037                               |
|              |      | HYPOTAURINE                  | 2.6505      | 0.0060198                 | 0.000770                               |
|              |      | DEOXYCYTIDINE                | 3.1045      | 0.0081909                 | 0.001238                               |
|              |      | CORTISOL 21-ACETATE          | 0.47683     | 0.0081909                 | 0.001238                               |
|              |      | THYMIDINE                    | 2.1614      | 0.0088629                 | 0.001443                               |
| B+R          | SD   | L-CYSTATHIONINE              | 22.796      | 1.43E-07                  | <0.000001                              |
|              |      | MANNITOL                     | 20.928      | 1.43E-07                  | <0.000001                              |
|              |      | ALPHA-D-GLUCOSE              | 4.3656      | 1.91E-07                  | <0.000001                              |
|              |      | OMEGA-HYDROXYDODECANOIC ACID | 2.8494      | 8.58E-07                  | <0.000001                              |

|  |  |                        |         |            |           |
|--|--|------------------------|---------|------------|-----------|
|  |  | 5-HYDROXYINDOLEACETATE | 0.37957 | 5.55E-06   | <0.000001 |
|  |  | FERULATE               | 0.2426  | 5.77E-05   | 0.000004  |
|  |  | STACHYOSE              | 3.4831  | 6.50E-05   | 0.000005  |
|  |  | 10-HYDROXYDECANOATE    | 0.45954 | 0.00015803 | 0.000015  |
|  |  | 3-HYDROXYBUTANOIC ACID | 0.25483 | 0.00022568 | 0.000024  |

**Table S4. Metabolites identified by fold-change analysis.** Fold-change analysis performed by Metaboanalyst. Direction of comparison was specified as fungal groups/B only. Test parameters were non-parametric with unequal variance and false discovery rate correction ( $p < 0.05$ ). Differences in normalized concentrations between B only and fungal colonized groups defined by Mann-Whitney test (two-sided) with false discovery rate correction.

| Cell population                                                                                                                                                                                        | Panel    | Markers                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Eosinophils                                                                                                                                                                                            | Myeloid  | CD45 <sup>+</sup> CD11b <sup>+</sup> SigF <sup>+</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| Neutrophils                                                                                                                                                                                            | Myeloid  | CD45 <sup>+</sup> CD11b <sup>+</sup> SigF <sup>+</sup> Ly6G <sup>+</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| Monocytes & macrophages<br>VAM1<br>VAM2<br>Monocytes<br>Int. macrophages<br>Macrophages<br>CD9 <sup>+</sup> macrophages<br>preVAM<br>CD11c <sup>+</sup> macrophages<br>CX3CR1 <sup>+</sup> macrophages | Myeloid  | CD45 <sup>+</sup> CD11b <sup>+</sup> SigF <sup>+</sup> Ly6G <sup>+</sup> CD64 <sup>+</sup><br>CD206 <sup>hi</sup> MHCII <sup>+</sup> Tim4 <sup>int</sup><br>CD206 <sup>hi</sup> MHCII <sup>-</sup> Tim4 <sup>hi</sup><br>CD206 <sup>lo/int</sup> Ly6C <sup>+</sup> MHCII <sup>-</sup><br>CD206 <sup>lo/int</sup> Ly6C <sup>+</sup> MHCII <sup>+</sup><br>CD206 <sup>lo/int</sup> Ly6C <sup>-</sup> MHCII <sup>+</sup><br>CD9 <sup>+</sup><br>CD11c <sup>-</sup><br>CD11c <sup>+</sup><br>CX3CR1 <sup>+</sup> |
| Dendritic cells<br>cDC1<br>cDC2<br>CX3CR1 <sup>+</sup> DC                                                                                                                                              | Myeloid  | CD45 <sup>+</sup> CD11b <sup>+</sup> SigF <sup>+</sup> Ly6G <sup>+</sup> CD64 <sup>+</sup> CD11c <sup>+</sup> MHCII <sup>+</sup><br>CD103 <sup>+</sup> CD11b <sup>-</sup><br>CD103 <sup>-</sup> CD11b <sup>+</sup><br>CX3CR1 <sup>+</sup>                                                                                                                                                                                                                                                                    |
| γδT cells                                                                                                                                                                                              | Lymphoid | CD45 <sup>+</sup> αβTCR <sup>-</sup> γδTCR <sup>+</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| αβTcells<br>CD8 <sup>+</sup><br>CD8 <sup>+</sup> NKT<br>CD8 <sup>+</sup> T cells<br>CD4 <sup>+</sup><br>CD4 <sup>+</sup> NKT<br>CD4 <sup>+</sup> Th<br>Th1<br>Th2<br>Th17<br>Treg                      | Lymphoid | CD45 <sup>+</sup> αβTCR <sup>-</sup> γδTCR <sup>-</sup><br>CD4 <sup>-</sup> CD8 <sup>+</sup><br>NK1.1 <sup>+</sup><br>NK1.1 <sup>-</sup><br>CD4 <sup>+</sup> CD8 <sup>-</sup><br>NK1.1 <sup>+</sup><br>NK1.1 <sup>-</sup><br>Tbet <sup>+</sup><br>GATA3 <sup>+</sup><br>RORγt <sup>+</sup> IL-17A <sup>+</sup><br>FOXP3 <sup>+</sup>                                                                                                                                                                         |
| B cells                                                                                                                                                                                                | Lymphoid | CD45 <sup>+</sup> αβTCR <sup>-</sup> γδTCR <sup>-</sup> CD19 <sup>+</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| TCR <sup>-</sup> CD19 <sup>-</sup><br>Tbet <sup>+</sup> ILC<br>ILC1<br>NK<br>ILC2<br>ILC3                                                                                                              | Lymphoid | CD45 <sup>+</sup> αβTCR <sup>-</sup> γδTCR <sup>-</sup> CD19 <sup>-</sup><br>CD90.2 <sup>+</sup> Tbet <sup>+</sup><br>NK1.1 <sup>+</sup> EOMES <sup>-</sup><br>NK1.1 <sup>+</sup> EOMES <sup>+</sup><br>CD90.2 <sup>+</sup> GATA3 <sup>+</sup><br>CD90.2 <sup>+</sup> RORγt <sup>+</sup>                                                                                                                                                                                                                     |

**Table S5. Immune cell characterization by extracellular and intracellular markers in flow cytometry panels.** VAM, vascular associated macrophage; Int, intermediate; DC, dendritic cell; cDC, conventional dendritic cell; NKT; natural killer T cell; Th, T helper cell; Treg, T regulatory cell; Lin, lineage; ILC, innate lymphoid cell; NK, natural killer cell.

| Cell type             | B        |          | B+C      |          | B+R      |          | B+M      |          |
|-----------------------|----------|----------|----------|----------|----------|----------|----------|----------|
|                       | Mean     | SD       | Mean     | SD       | Mean     | SD       | Mean     | SD       |
| <b>Th1</b>            | 6.64E+02 | 5.59E+02 | 3.28E+03 | 2.23E+03 | 8.97E+02 | 7.53E+02 | 9.27E+02 | 1.27E+03 |
| <b>Th2</b>            | 3.09E+03 | 2.17E+03 | 3.80E+03 | 2.45E+03 | 1.82E+03 | 1.32E+03 | 2.09E+03 | 2.09E+03 |
| <b>Th17</b>           | 5.92E+02 | 4.86E+02 | 1.82E+03 | 7.05E+02 | 3.54E+02 | 1.81E+02 | 2.82E+02 | 3.45E+02 |
| <b>Tregs</b>          | 1.70E+03 | 9.70E+02 | 1.62E+03 | 5.31E+02 | 1.15E+03 | 9.41E+02 | 9.21E+02 | 9.24E+02 |
| <b>CD8+ T cells</b>   | 3.05E+03 | 1.98E+03 | 5.70E+03 | 1.81E+03 | 2.23E+03 | 1.82E+03 | 1.29E+03 | 6.97E+02 |
| <b>B cells</b>        | 5.03E+04 | 4.58E+04 | 1.29E+05 | 7.29E+04 | 4.97E+04 | 3.89E+04 | 1.77E+04 | 1.10E+04 |
| <b>γδT cells</b>      | 4.65E+03 | 3.14E+03 | 1.66E+04 | 4.71E+03 | 4.91E+03 | 3.26E+03 | 2.76E+03 | 1.86E+03 |
| <b>CD4+ NKT cells</b> | 2.93E+02 | 2.25E+02 | 4.53E+02 | 1.63E+02 | 2.52E+02 | 2.31E+02 | 3.16E+02 | 2.88E+02 |
| <b>CD8+ NKT cells</b> | 3.10E+02 | 2.66E+02 | 6.75E+02 | 4.53E+02 | 2.97E+02 | 2.99E+02 | 2.81E+02 | 2.46E+02 |
| <b>ILC1</b>           | 2.47E+03 | 2.19E+03 | 8.43E+03 | 3.79E+03 | 1.96E+03 | 1.54E+03 | 1.01E+03 | 7.00E+02 |
| <b>NK</b>             | 2.61E+03 | 2.02E+03 | 6.73E+03 | 3.89E+03 | 1.45E+03 | 9.93E+02 | 1.77E+03 | 1.41E+03 |
| <b>ILC2</b>           | 1.39E+04 | 9.65E+03 | 1.98E+04 | 2.51E+03 | 1.20E+04 | 8.14E+03 | 6.11E+03 | 2.67E+03 |
| <b>ILC3</b>           | 1.96E+03 | 1.01E+03 | 3.99E+03 | 1.99E+03 | 1.86E+03 | 1.34E+03 | 1.16E+03 | 9.64E+02 |
| <b>CD64+</b>          | 1.45E+05 | 1.18E+05 | 3.01E+05 | 1.11E+05 | 1.67E+05 | 8.72E+04 | 9.56E+04 | 5.56E+04 |
| <b>Monocytes</b>      | 1.44E+03 | 1.27E+03 | 9.54E+02 | 3.74E+02 | 1.78E+03 | 1.52E+03 | 1.28E+03 | 8.47E+02 |
| <b>Int. mφ</b>        | 4.13E+03 | 3.56E+03 | 1.08E+04 | 4.67E+03 | 5.18E+03 | 3.17E+03 | 2.32E+03 | 1.58E+03 |
| <b>CD11c+ mφ</b>      | 1.18E+04 | 1.03E+04 | 1.35E+04 | 4.35E+03 | 1.48E+04 | 8.09E+03 | 1.24E+04 | 9.36E+03 |
| <b>CX3CR1+ mφ</b>     | 1.61E+04 | 1.24E+04 | 2.72E+04 | 9.65E+03 | 2.02E+04 | 1.10E+04 | 1.39E+04 | 9.61E+03 |
| <b>CD9+ mφ</b>        | 1.56E+04 | 1.22E+04 | 1.73E+04 | 4.84E+03 | 1.78E+04 | 8.56E+03 | 1.65E+04 | 1.14E+04 |
| <b>preVAM</b>         | 1.51E+04 | 9.78E+03 | 3.67E+04 | 1.41E+04 | 2.50E+04 | 1.26E+04 | 1.35E+04 | 7.95E+03 |
| <b>VAM1</b>           | 2.87E+04 | 2.11E+04 | 1.01E+05 | 4.33E+04 | 4.03E+04 | 2.17E+04 | 1.93E+04 | 1.18E+04 |
| <b>VAM2</b>           | 1.64E+04 | 1.18E+04 | 4.32E+04 | 1.52E+04 | 2.38E+04 | 1.66E+04 | 1.19E+04 | 8.69E+03 |
| <b>cDC1</b>           | 4.62E+03 | 2.97E+03 | 1.05E+04 | 3.86E+03 | 4.05E+03 | 2.54E+03 | 4.34E+03 | 3.93E+03 |
| <b>cDC2</b>           | 6.14E+04 | 4.52E+04 | 8.52E+04 | 2.15E+04 | 6.55E+04 | 3.28E+04 | 4.94E+04 | 3.26E+04 |
| <b>CX3CR1+ DC</b>     | 1.18E+04 | 7.26E+03 | 2.14E+04 | 7.38E+03 | 1.13E+04 | 5.51E+03 | 1.18E+04 | 7.96E+03 |
| <b>Eosinophils</b>    | 8.25E+04 | 5.07E+04 | 1.05E+05 | 2.55E+04 | 9.37E+04 | 4.19E+04 | 3.67E+04 | 2.26E+04 |
| <b>Neutrophils</b>    | 9.54E+03 | 5.46E+03 | 3.11E+04 | 1.89E+04 | 1.07E+04 | 4.63E+03 | 6.49E+03 | 4.04E+03 |

**Table S6. WAT immune cell summary statistics in males fed SD.** VAM, vascular associated macrophage; Int, intermediate; DC, dendritic cell; cDC, conventional dendritic cell; NKT; natural killer T cell; Th, T helper cell; Treg, T regulatory cell; ILC, innate lymphoid cell; NK, natural killer cell; mφ, macrophage.

| Cell type                               | B        |          | B+C      |          | B+R      |          | B+M      |          |
|-----------------------------------------|----------|----------|----------|----------|----------|----------|----------|----------|
|                                         | Mean     | SD       | Mean     | SD       | Mean     | SD       | Mean     | SD       |
| <b>Th1</b>                              | 1.25E+03 | 6.88E+02 | 1.41E+03 | 8.86E+02 | 1.08E+03 | 1.06E+03 | 6.34E+02 | 4.22E+02 |
| <b>Th2</b>                              | 2.95E+03 | 2.09E+03 | 2.39E+03 | 1.75E+03 | 1.40E+03 | 8.03E+02 | 1.66E+03 | 8.33E+02 |
| <b>Th17</b>                             | 7.02E+02 | 4.49E+02 | 1.11E+03 | 6.68E+02 | 5.64E+02 | 5.30E+02 | 3.93E+02 | 3.70E+02 |
| <b>Tregs</b>                            | 6.29E+02 | 4.49E+02 | 6.00E+02 | 1.94E+02 | 3.62E+02 | 1.65E+02 | 3.30E+02 | 1.93E+02 |
| <b>CD8+ T cells</b>                     | 2.43E+03 | 1.40E+03 | 3.32E+03 | 7.12E+02 | 1.63E+03 | 7.73E+02 | 1.69E+03 | 1.21E+03 |
| <b>B cells</b>                          | 6.32E+04 | 5.15E+04 | 1.11E+05 | 4.20E+04 | 4.61E+04 | 2.79E+04 | 1.18E+04 | 8.32E+03 |
| <b><math>\gamma\delta</math>T cells</b> | 2.23E+03 | 1.03E+03 | 7.00E+03 | 2.32E+03 | 3.89E+03 | 2.52E+03 | 1.43E+03 | 8.84E+02 |
| <b>CD4+ NKT cells</b>                   | 2.59E+02 | 1.48E+02 | 2.74E+02 | 1.01E+02 | 2.94E+02 | 1.83E+02 | 1.54E+02 | 6.78E+01 |
| <b>CD8+ NKT cells</b>                   | 1.19E+02 | 8.33E+01 | 1.89E+02 | 8.33E+01 | 2.07E+02 | 2.15E+02 | 4.81E+01 | 4.75E+01 |
| <b>ILC1</b>                             | 6.74E+02 | 6.17E+02 | 2.29E+03 | 1.30E+03 | 1.38E+03 | 1.08E+03 | 3.09E+02 | 8.69E+01 |
| <b>NK</b>                               | 6.88E+02 | 6.87E+02 | 2.04E+03 | 1.43E+03 | 8.47E+02 | 7.43E+02 | 3.35E+02 | 1.88E+02 |
| <b>ILC2</b>                             | 1.35E+04 | 6.53E+03 | 1.70E+04 | 6.10E+03 | 2.02E+04 | 5.85E+03 | 6.87E+03 | 3.16E+03 |
| <b>ILC3</b>                             | 1.79E+03 | 8.51E+02 | 3.55E+03 | 2.49E+03 | 3.13E+03 | 2.01E+03 | 7.41E+02 | 3.80E+02 |
| <b>CD64+</b>                            | 1.41E+05 | 8.10E+04 | 3.18E+05 | 1.79E+05 | 2.83E+05 | 1.58E+05 | 7.78E+04 | 3.14E+04 |
| <b>Monocytes</b>                        | 5.42E+02 | 3.83E+02 | 1.19E+03 | 1.02E+03 | 3.84E+02 | 1.47E+02 | 4.99E+02 | 2.74E+02 |
| <b>Int. m<math>\phi</math></b>          | 3.03E+03 | 2.58E+03 | 1.28E+04 | 9.19E+03 | 5.02E+03 | 5.02E+03 | 1.97E+03 | 8.64E+02 |
| <b>CD11c+ m<math>\phi</math></b>        | 3.66E+03 | 1.90E+03 | 6.32E+03 | 3.75E+03 | 7.34E+03 | 2.61E+03 | 6.23E+03 | 6.88E+03 |
| <b>CX3CR1+ m<math>\phi</math></b>       | 1.34E+04 | 7.77E+03 | 2.95E+04 | 1.96E+04 | 3.07E+04 | 2.29E+04 | 1.49E+04 | 9.02E+03 |
| <b>CD9+ m<math>\phi</math></b>          | 6.93E+03 | 4.02E+03 | 1.25E+04 | 9.93E+03 | 1.07E+04 | 4.23E+03 | 1.30E+04 | 5.79E+03 |
| <b>preVAM</b>                           | 2.33E+04 | 1.35E+04 | 3.33E+04 | 2.28E+04 | 4.68E+04 | 2.67E+04 | 1.62E+04 | 8.77E+03 |
| <b>VAM1</b>                             | 4.55E+04 | 3.06E+04 | 1.24E+05 | 7.28E+04 | 8.95E+04 | 5.33E+04 | 1.66E+04 | 7.88E+03 |
| <b>VAM2</b>                             | 1.58E+04 | 1.17E+04 | 3.30E+04 | 2.85E+04 | 3.99E+04 | 2.15E+04 | 5.51E+03 | 3.13E+03 |
| <b>cDC1</b>                             | 2.47E+03 | 1.55E+03 | 5.69E+03 | 3.03E+03 | 4.26E+03 | 2.23E+03 | 8.61E+02 | 2.91E+02 |
| <b>cDC2</b>                             | 4.55E+04 | 2.55E+04 | 8.08E+04 | 3.63E+04 | 8.17E+04 | 3.88E+04 | 2.17E+04 | 1.70E+04 |
| <b>CX3CR1+ DC</b>                       | 9.71E+03 | 3.81E+03 | 2.57E+04 | 1.05E+04 | 1.29E+04 | 5.89E+03 | 8.30E+03 | 4.10E+03 |
| <b>Eosinophils</b>                      | 1.41E+05 | 8.90E+04 | 1.90E+05 | 5.18E+04 | 2.48E+05 | 7.88E+04 | 4.83E+04 | 2.38E+04 |
| <b>Neutrophils</b>                      | 5.90E+03 | 3.74E+03 | 1.73E+04 | 8.37E+03 | 1.08E+04 | 5.93E+03 | 2.26E+03 | 1.07E+03 |

**Table S7. WAT immune cell summary statistics in females fed SD.** VAM, vascular associated macrophage; Int, intermediate; DC, dendritic cell; cDC, conventional dendritic cell; NKT; natural killer T cell; Th, T helper cell; Treg, T regulatory cell; ILC, innate lymphoid cell; NK, natural killer cell; m $\phi$ , macrophage.

| Cell type              | B        |          | B+C      |          | B+R      |          | B+M      |          |
|------------------------|----------|----------|----------|----------|----------|----------|----------|----------|
|                        | Mean     | SD       | Mean     | SD       | Mean     | SD       | Mean     | SD       |
| Th1                    | 1.60E+06 | 9.87E+05 | 8.37E+06 | 4.33E+06 | 4.31E+06 | 2.97E+06 | 2.80E+06 | 2.41E+06 |
| Th2                    | 1.56E+07 | 4.39E+06 | 1.75E+07 | 8.22E+06 | 1.37E+07 | 4.18E+06 | 1.50E+07 | 1.03E+07 |
| Th17                   | 1.70E+06 | 7.16E+05 | 4.19E+06 | 5.60E+05 | 1.70E+06 | 4.81E+05 | 2.02E+06 | 1.52E+06 |
| Tregs                  | 1.73E+07 | 2.98E+06 | 1.95E+07 | 2.96E+06 | 1.46E+07 | 2.26E+06 | 1.45E+07 | 6.45E+06 |
| CD8+ T cells           | 8.20E+07 | 1.57E+07 | 9.75E+07 | 2.96E+07 | 6.24E+07 | 1.27E+07 | 7.04E+07 | 3.05E+07 |
| B cells                | 5.91E+08 | 8.26E+07 | 5.78E+08 | 6.27E+07 | 5.04E+08 | 1.00E+08 | 4.99E+08 | 2.52E+08 |
| $\gamma\delta$ T cells | 1.17E+07 | 1.58E+06 | 1.35E+07 | 1.36E+06 | 9.18E+06 | 1.73E+06 | 1.06E+07 | 4.20E+06 |
| CD4+ NKT cells         | 1.39E+06 | 3.06E+05 | 1.62E+06 | 3.99E+05 | 1.17E+06 | 2.20E+05 | 9.39E+05 | 4.03E+05 |
| CD8+ NKT cells         | 4.57E+05 | 1.03E+05 | 8.40E+05 | 1.73E+05 | 5.89E+05 | 2.61E+05 | 3.61E+05 | 1.91E+05 |
| ILC1                   | 9.73E+04 | 8.98E+04 | 3.35E+05 | 3.29E+05 | 2.39E+04 | 3.38E+04 | 3.44E+05 | 3.97E+05 |
| NK                     | 4.10E+05 | 3.73E+05 | 2.16E+06 | 2.33E+06 | 2.08E+05 | 2.29E+05 | 2.15E+06 | 2.59E+06 |
| ILC2                   | 2.50E+06 | 5.60E+05 | 6.45E+06 | 2.55E+06 | 2.68E+06 | 1.13E+06 | 2.43E+06 | 1.73E+06 |
| ILC3                   | 8.31E+05 | 1.85E+05 | 1.57E+06 | 2.78E+05 | 8.11E+05 | 2.50E+05 | 6.81E+05 | 4.47E+05 |
| CD64+                  | 2.53E+06 | 1.06E+06 | 1.58E+06 | 6.02E+05 | 1.33E+06 | 9.48E+05 | 1.71E+06 | 1.01E+06 |
| cDC1                   | 4.73E+05 | 3.24E+05 | 6.88E+05 | 2.75E+05 | 5.01E+05 | 2.82E+05 | 3.32E+05 | 1.57E+05 |
| cDC2                   | 1.13E+07 | 3.58E+06 | 1.37E+07 | 6.17E+06 | 6.97E+06 | 2.38E+06 | 1.00E+07 | 2.44E+06 |
| CX3CR1+ DC             | 9.63E+06 | 4.06E+06 | 7.15E+06 | 4.81E+05 | 4.67E+06 | 1.48E+06 | 9.40E+06 | 4.06E+06 |
| Eosinophils            | 6.14E+06 | 2.52E+06 | 6.69E+06 | 2.12E+06 | 2.98E+06 | 1.41E+06 | 6.02E+06 | 2.30E+06 |
| Neutrophils            | 3.32E+07 | 1.29E+07 | 3.83E+07 | 8.82E+06 | 2.43E+07 | 1.09E+07 | 2.89E+07 | 1.17E+07 |

**Table S8. Spleen immune cell summary statistics in males fed SD.** VAM, vascular associated macrophage; Int, intermediate; DC, dendritic cell; cDC, conventional dendritic cell; NKT; natural killer T cell; Th, T helper cell; Treg, T regulatory cell; ILC, innate lymphoid cell; NK, natural killer cell.

| Cell type              | B        |          | B+C      |          | B+R      |          | B+M      |          |
|------------------------|----------|----------|----------|----------|----------|----------|----------|----------|
|                        | Mean     | SD       | Mean     | SD       | Mean     | SD       | Mean     | SD       |
| Th1                    | 1.96E+06 | 8.13E+05 | 6.43E+06 | 4.53E+06 | 5.04E+06 | 1.74E+06 | 1.93E+06 | 1.16E+06 |
| Th2                    | 1.68E+07 | 7.42E+06 | 1.64E+07 | 6.84E+06 | 1.23E+07 | 2.36E+06 | 1.72E+07 | 1.05E+07 |
| Th17                   | 1.63E+06 | 5.26E+05 | 4.74E+06 | 1.71E+06 | 2.29E+06 | 6.38E+05 | 1.63E+06 | 8.04E+05 |
| Tregs                  | 1.53E+07 | 2.98E+06 | 2.09E+07 | 5.03E+06 | 1.54E+07 | 2.60E+06 | 1.76E+07 | 6.12E+06 |
| CD8+ T cells           | 7.59E+07 | 2.76E+07 | 9.16E+07 | 1.98E+07 | 7.58E+07 | 1.45E+07 | 8.28E+07 | 2.40E+07 |
| B cells                | 4.94E+08 | 9.39E+07 | 6.27E+08 | 1.57E+08 | 5.46E+08 | 6.40E+07 | 5.20E+08 | 1.55E+08 |
| $\gamma\delta$ T cells | 1.30E+07 | 3.16E+06 | 1.72E+07 | 3.73E+06 | 1.41E+07 | 2.53E+06 | 1.50E+07 | 4.08E+06 |
| CD4+ NKT cells         | 1.85E+06 | 5.51E+05 | 2.29E+06 | 5.86E+05 | 1.96E+06 | 4.64E+05 | 2.12E+06 | 5.75E+05 |
| CD8+ NKT cells         | 4.88E+05 | 2.61E+05 | 7.21E+05 | 2.27E+05 | 6.95E+05 | 1.22E+05 | 3.88E+05 | 1.47E+05 |
| ILC1                   | 1.65E+05 | 9.37E+04 | 2.53E+05 | 2.37E+05 | 1.29E+05 | 1.03E+05 | 2.49E+05 | 2.88E+05 |
| NK                     | 7.02E+05 | 5.66E+05 | 9.32E+05 | 7.31E+05 | 5.35E+05 | 3.12E+05 | 1.11E+06 | 1.32E+06 |
| ILC2                   | 1.95E+06 | 4.46E+05 | 3.85E+06 | 1.77E+06 | 3.59E+06 | 3.83E+05 | 2.97E+06 | 1.67E+06 |
| ILC3                   | 8.09E+05 | 2.29E+05 | 1.24E+06 | 3.33E+05 | 1.31E+06 | 2.21E+05 | 8.68E+05 | 3.82E+05 |
| CD64+                  | 1.47E+06 | 8.64E+05 | 2.18E+06 | 9.87E+05 | 1.83E+06 | 1.19E+06 | 1.49E+06 | 7.35E+05 |
| cDC1                   | 3.92E+05 | 2.57E+05 | 7.20E+05 | 3.24E+05 | 5.02E+05 | 1.47E+05 | 5.01E+05 | 2.36E+05 |
| cDC2                   | 7.17E+06 | 3.71E+06 | 1.13E+07 | 3.33E+06 | 7.07E+06 | 7.86E+05 | 8.04E+06 | 1.89E+06 |
| CX3CR1+ DC             | 5.33E+06 | 3.09E+06 | 6.85E+06 | 1.82E+06 | 4.70E+06 | 2.96E+05 | 7.07E+06 | 1.45E+06 |
| Eosinophils            | 2.50E+06 | 1.02E+06 | 4.07E+06 | 1.35E+06 | 2.85E+06 | 7.17E+05 | 3.36E+06 | 1.02E+06 |
| Neutrophils            | 1.44E+07 | 3.92E+06 | 2.11E+07 | 4.63E+06 | 1.74E+07 | 3.51E+06 | 1.39E+07 | 3.65E+06 |

**Table S9. Spleen immune cell summary statistics in females fed SD.** VAM, vascular associated macrophage; Int, intermediate; DC, dendritic cell; cDC, conventional dendritic cell; NKT; natural killer T cell; Th, T helper cell; Treg, T regulatory cell; ILC, innate lymphoid cell; NK, natural killer cell.

| Cell type      | B        |          | B+C      |          | B+R      |          | B+M      |          |
|----------------|----------|----------|----------|----------|----------|----------|----------|----------|
|                | Mean     | SD       | Mean     | SD       | Mean     | SD       | Mean     | SD       |
| Th1            | 1.10E+03 | 6.81E+02 | 2.55E+03 | 1.85E+03 | 8.70E+02 | 3.50E+02 | 5.24E+02 | 5.72E+02 |
| Th2            | 4.36E+03 | 2.74E+03 | 6.13E+03 | 3.56E+03 | 3.11E+03 | 1.53E+03 | 3.62E+03 | 2.68E+03 |
| Th17           | 1.17E+03 | 7.67E+02 | 4.03E+03 | 1.58E+03 | 1.49E+03 | 6.61E+02 | 2.62E+02 | 2.44E+02 |
| Tregs          | 3.33E+03 | 2.37E+03 | 4.14E+03 | 1.50E+03 | 2.40E+03 | 6.08E+02 | 2.70E+03 | 2.51E+03 |
| CD8+ T cells   | 3.65E+03 | 2.21E+03 | 7.37E+03 | 3.03E+03 | 4.42E+03 | 2.34E+03 | 2.61E+03 | 1.99E+03 |
| B cells        | 2.13E+04 | 1.70E+04 | 5.18E+04 | 2.18E+04 | 3.05E+04 | 1.33E+04 | 1.10E+04 | 9.71E+03 |
| γδT cells      | 4.76E+03 | 2.11E+03 | 1.67E+04 | 7.24E+03 | 8.03E+03 | 1.99E+03 | 4.14E+03 | 2.45E+03 |
| CD4+ NKT cells | 2.76E+02 | 1.89E+02 | 3.01E+02 | 1.46E+02 | 1.44E+02 | 6.68E+01 | 3.35E+02 | 2.72E+02 |
| CD8+ NKT cells | 4.97E+02 | 4.53E+02 | 7.59E+02 | 4.22E+02 | 3.70E+02 | 1.40E+02 | 3.11E+02 | 2.28E+02 |
| ILC1           | 1.92E+03 | 1.38E+03 | 5.26E+03 | 3.89E+03 | 3.00E+03 | 2.45E+03 | 1.39E+03 | 8.89E+02 |
| NK             | 2.36E+03 | 1.87E+03 | 5.82E+03 | 4.93E+03 | 2.31E+03 | 1.10E+03 | 2.18E+03 | 1.34E+03 |
| ILC2           | 8.52E+03 | 3.77E+03 | 1.68E+04 | 6.91E+03 | 1.33E+04 | 3.85E+03 | 7.59E+03 | 4.21E+03 |
| ILC3           | 2.33E+03 | 1.28E+03 | 2.98E+03 | 8.54E+02 | 3.57E+03 | 1.22E+03 | 1.32E+03 | 1.15E+03 |
| CD64+          | 1.49E+05 | 8.69E+04 | 1.86E+05 | 4.10E+04 | 2.20E+05 | 6.64E+04 | 1.30E+05 | 6.46E+04 |
| Monocytes      | 3.11E+03 | 2.33E+03 | 2.89E+03 | 2.20E+03 | 3.35E+03 | 1.41E+03 | 1.78E+03 | 4.28E+02 |
| Int. mφ        | 5.03E+03 | 3.98E+03 | 6.63E+03 | 2.03E+03 | 6.68E+03 | 1.86E+03 | 4.98E+03 | 3.34E+03 |
| CD11c+ mφ      | 2.05E+04 | 1.30E+04 | 1.45E+04 | 3.94E+03 | 3.17E+04 | 1.84E+04 | 2.30E+04 | 1.37E+04 |
| CX3CR1+ mφ     | 2.06E+04 | 1.32E+04 | 1.98E+04 | 8.46E+03 | 2.89E+04 | 1.33E+04 | 1.90E+04 | 1.06E+04 |
| CD9+ mφ        | 2.36E+04 | 1.63E+04 | 1.73E+04 | 2.28E+03 | 3.62E+04 | 1.54E+04 | 2.27E+04 | 1.13E+04 |
| preVAM         | 1.71E+04 | 9.13E+03 | 2.23E+04 | 8.75E+03 | 2.23E+04 | 6.15E+03 | 1.69E+04 | 8.75E+03 |
| VAM1           | 2.66E+04 | 1.73E+04 | 4.71E+04 | 1.18E+04 | 4.11E+04 | 1.10E+04 | 2.11E+04 | 9.53E+03 |
| VAM2           | 1.83E+04 | 1.17E+04 | 2.32E+04 | 7.50E+03 | 2.89E+04 | 7.16E+03 | 1.31E+04 | 8.09E+03 |
| cDC1           | 3.97E+03 | 2.29E+03 | 1.10E+04 | 7.66E+03 | 5.49E+03 | 2.94E+03 | 3.60E+03 | 2.06E+03 |
| cDC2           | 6.25E+04 | 3.48E+04 | 7.38E+04 | 2.32E+04 | 8.30E+04 | 1.63E+04 | 5.47E+04 | 2.42E+04 |
| CX3CR1+ DC     | 1.25E+04 | 5.53E+03 | 1.70E+04 | 5.44E+03 | 1.57E+04 | 2.71E+03 | 1.32E+04 | 5.91E+03 |
| Eosinophils    | 8.18E+04 | 4.52E+04 | 1.90E+05 | 1.06E+05 | 1.00E+05 | 1.83E+04 | 6.54E+04 | 4.08E+04 |
| Neutrophils    | 8.62E+03 | 4.04E+03 | 1.99E+04 | 1.15E+04 | 1.04E+04 | 5.13E+03 | 8.73E+03 | 5.33E+03 |

**Table S10. WAT immune cell summary statistics in males fed HFHS.** VAM, vascular associated macrophage; Int, intermediate; DC, dendritic cell; cDC, conventional dendritic cell; NKT; natural killer T cell; Th, T helper cell; Treg, T regulatory cell; ILC, innate lymphoid cell; NK, natural killer cell; mφ, macrophage.

| Cell type              | B        |          | B+C      |          | B+R      |          | B+M      |          |
|------------------------|----------|----------|----------|----------|----------|----------|----------|----------|
|                        | Mean     | SD       | Mean     | SD       | Mean     | SD       | Mean     | SD       |
| Th1                    | 1.09E+03 | 9.06E+02 | 2.28E+03 | 2.55E+03 | 6.72E+02 | 2.24E+02 | 3.03E+02 | 2.24E+02 |
| Th2                    | 2.07E+03 | 1.68E+03 | 2.32E+03 | 1.47E+03 | 1.77E+03 | 5.31E+02 | 2.82E+03 | 2.42E+03 |
| Th17                   | 5.93E+02 | 4.43E+02 | 1.39E+03 | 9.40E+02 | 7.92E+02 | 3.52E+02 | 8.81E+01 | 7.08E+01 |
| Tregs                  | 5.08E+02 | 4.47E+02 | 6.96E+02 | 5.87E+02 | 5.58E+02 | 1.68E+02 | 3.98E+02 | 2.89E+02 |
| CD8+ T cells           | 2.06E+03 | 1.58E+03 | 3.45E+03 | 3.00E+03 | 2.63E+03 | 6.83E+02 | 2.49E+03 | 1.79E+03 |
| B cells                | 1.56E+04 | 1.21E+04 | 2.37E+04 | 1.73E+04 | 2.20E+04 | 1.05E+04 | 1.11E+04 | 8.26E+03 |
| $\gamma\delta$ T cells | 2.74E+03 | 2.18E+03 | 4.83E+03 | 3.87E+03 | 2.92E+03 | 7.90E+02 | 1.83E+03 | 1.04E+03 |
| CD4+ NKT cells         | 1.75E+02 | 1.60E+02 | 1.70E+02 | 1.51E+02 | 1.60E+02 | 4.78E+01 | 2.26E+02 | 1.52E+02 |
| CD8+ NKT cells         | 1.11E+02 | 9.82E+01 | 3.25E+02 | 3.01E+02 | 2.11E+02 | 1.85E+02 | 1.14E+02 | 7.60E+01 |
| ILC1                   | 7.22E+02 | 6.70E+02 | 1.30E+03 | 1.18E+03 | 5.11E+02 | 2.09E+02 | 5.12E+02 | 4.89E+02 |
| NK                     | 4.78E+02 | 4.44E+02 | 1.20E+03 | 1.13E+03 | 3.95E+02 | 1.68E+02 | 3.39E+02 | 2.88E+02 |
| ILC2                   | 1.10E+04 | 8.16E+03 | 1.04E+04 | 6.73E+03 | 1.34E+04 | 4.48E+03 | 7.62E+03 | 5.27E+03 |
| ILC3                   | 1.66E+03 | 1.29E+03 | 1.38E+03 | 1.11E+03 | 1.87E+03 | 4.53E+02 | 6.59E+02 | 3.71E+02 |
| CD64+                  | 1.22E+05 | 8.53E+04 | 7.14E+04 | 2.87E+04 | 1.83E+05 | 1.17E+05 | 9.71E+04 | 5.89E+04 |
| Monocytes              | 6.69E+02 | 4.35E+02 | 4.89E+02 | 3.57E+02 | 7.30E+02 | 3.41E+02 | 1.13E+03 | 9.08E+02 |
| Int. m $\phi$          | 1.56E+03 | 1.32E+03 | 1.34E+03 | 7.78E+02 | 1.96E+03 | 9.45E+02 | 2.32E+03 | 2.27E+03 |
| CD11c+ m $\phi$        | 6.80E+03 | 5.56E+03 | 3.12E+03 | 1.58E+03 | 7.35E+03 | 2.98E+03 | 7.38E+03 | 5.64E+03 |
| CX3CR1+ m $\phi$       | 1.50E+04 | 1.15E+04 | 7.04E+03 | 4.03E+03 | 1.64E+04 | 5.11E+03 | 1.71E+04 | 1.07E+04 |
| CD9+ m $\phi$          | 9.62E+03 | 7.61E+03 | 3.07E+03 | 8.25E+02 | 1.74E+04 | 1.26E+04 | 1.46E+04 | 9.17E+03 |
| preVAM                 | 2.27E+04 | 1.70E+04 | 1.33E+04 | 8.55E+03 | 2.87E+04 | 1.84E+04 | 2.03E+04 | 1.27E+04 |
| VAM1                   | 2.67E+04 | 2.07E+04 | 2.41E+04 | 1.70E+04 | 4.30E+04 | 2.29E+04 | 1.94E+04 | 1.37E+04 |
| VAM2                   | 1.39E+04 | 1.02E+04 | 1.19E+04 | 8.70E+03 | 1.60E+04 | 1.35E+04 | 8.67E+03 | 5.38E+03 |
| cDC1                   | 1.88E+03 | 1.15E+03 | 3.03E+03 | 1.07E+03 | 2.69E+03 | 1.69E+03 | 2.38E+03 | 1.60E+03 |
| cDC2                   | 4.06E+04 | 2.54E+04 | 2.55E+04 | 8.00E+03 | 6.82E+04 | 3.77E+04 | 2.93E+04 | 1.81E+04 |
| CX3CR1+ DC             | 1.01E+04 | 7.52E+03 | 1.01E+04 | 4.46E+03 | 1.27E+04 | 4.09E+03 | 1.08E+04 | 5.98E+03 |
| Eosinophils            | 1.62E+05 | 1.07E+05 | 1.87E+05 | 1.27E+05 | 2.59E+05 | 1.36E+05 | 8.81E+04 | 5.45E+04 |
| Neutrophils            | 5.73E+03 | 3.49E+03 | 1.45E+04 | 5.26E+03 | 6.31E+03 | 3.24E+03 | 2.79E+03 | 1.27E+03 |

**Table S11. WAT immune cell summary statistics in females fed HFHS.** VAM, vascular associated macrophage; Int, intermediate; DC, dendritic cell; cDC, conventional dendritic cell; NKT; natural killer T cell; Th, T helper cell; Treg, T regulatory cell; ILC, innate lymphoid cell; NK, natural killer cell; m $\phi$ , macrophage.

| Cell type              | B        |          | B+C      |          | B+R      |          | B+M      |          |
|------------------------|----------|----------|----------|----------|----------|----------|----------|----------|
|                        | Mean     | SD       | Mean     | SD       | Mean     | SD       | Mean     | SD       |
| Th1                    | 3.39E+06 | 2.29E+06 | 6.28E+06 | 4.54E+06 | 2.05E+06 | 6.35E+05 | 9.85E+05 | 3.46E+05 |
| Th2                    | 1.62E+07 | 6.46E+06 | 2.13E+07 | 9.31E+06 | 1.14E+07 | 4.23E+06 | 8.10E+06 | 3.26E+06 |
| Th17                   | 2.19E+06 | 1.02E+06 | 5.60E+06 | 1.00E+06 | 2.18E+06 | 5.04E+05 | 9.22E+05 | 2.67E+05 |
| Tregs                  | 1.92E+07 | 4.72E+06 | 2.26E+07 | 3.63E+06 | 1.67E+07 | 2.86E+06 | 1.35E+07 | 6.44E+06 |
| CD8+ T cells           | 8.66E+07 | 2.36E+07 | 8.68E+07 | 1.67E+07 | 7.36E+07 | 1.90E+07 | 6.15E+07 | 2.56E+07 |
| B cells                | 6.64E+08 | 1.51E+08 | 6.37E+08 | 1.00E+08 | 6.07E+08 | 1.15E+08 | 4.85E+08 | 2.39E+08 |
| $\gamma\delta$ T cells | 1.46E+07 | 3.48E+06 | 1.46E+07 | 2.51E+06 | 1.26E+07 | 2.45E+06 | 1.07E+07 | 4.66E+06 |
| CD4+ NKT cells         | 1.16E+06 | 4.52E+05 | 1.08E+06 | 3.24E+05 | 1.00E+06 | 2.33E+05 | 8.94E+05 | 5.16E+05 |
| CD8+ NKT cells         | 6.35E+05 | 2.32E+05 | 8.65E+05 | 2.65E+05 | 5.88E+05 | 2.56E+05 | 3.62E+05 | 1.62E+05 |
| ILC1                   | 3.60E+05 | 2.53E+05 | 6.52E+05 | 2.73E+05 | 1.84E+05 | 7.56E+04 | 1.05E+05 | 8.42E+04 |
| NK                     | 1.09E+06 | 8.11E+05 | 2.69E+06 | 1.21E+06 | 5.10E+05 | 1.69E+05 | 2.32E+05 | 1.84E+05 |
| ILC2                   | 3.14E+06 | 1.29E+06 | 3.93E+06 | 1.71E+06 | 2.33E+06 | 6.97E+05 | 1.48E+06 | 7.70E+05 |
| ILC3                   | 9.79E+05 | 3.63E+05 | 1.23E+06 | 2.84E+05 | 9.77E+05 | 3.69E+05 | 5.27E+05 | 2.97E+05 |
| CD64+                  | 2.60E+06 | 1.58E+06 | 1.39E+06 | 2.30E+05 | 1.78E+06 | 8.00E+05 | 1.33E+06 | 3.96E+05 |
| cDC1                   | 2.21E+05 | 1.22E+05 | 3.84E+05 | 3.42E+05 | 1.08E+05 | 2.25E+04 | 2.61E+05 | 1.89E+05 |
| cDC2                   | 1.33E+07 | 5.52E+06 | 1.01E+07 | 2.70E+06 | 1.04E+07 | 3.68E+06 | 1.34E+07 | 3.62E+06 |
| CX3CR1+ DC             | 1.03E+07 | 4.27E+06 | 6.05E+06 | 1.32E+06 | 6.90E+06 | 2.22E+06 | 1.03E+07 | 3.08E+06 |
| Eosinophils            | 5.77E+06 | 2.12E+06 | 4.17E+06 | 1.53E+06 | 4.23E+06 | 1.98E+06 | 5.53E+06 | 1.78E+06 |
| Neutrophils            | 2.64E+07 | 7.92E+06 | 2.01E+07 | 4.59E+06 | 1.95E+07 | 9.69E+06 | 2.21E+07 | 5.26E+06 |

**Table S12. Spleen immune cell summary statistics in males fed HFHS.** VAM, vascular associated macrophage; Int, intermediate; DC, dendritic cell; cDC, conventional dendritic cell; NKT; natural killer T cell; Th, T helper cell; Treg, T regulatory cell; ILC, innate lymphoid cell; NK, natural killer cell.

| Cell type              | B        |          | B+C      |          | B+R      |          | B+M      |          |
|------------------------|----------|----------|----------|----------|----------|----------|----------|----------|
|                        | Mean     | SD       | Mean     | SD       | Mean     | SD       | Mean     | SD       |
| Th1                    | 2.98E+06 | 1.84E+06 | 6.57E+06 | 4.49E+06 | 2.55E+06 | 6.45E+05 | 5.72E+06 | 4.83E+06 |
| Th2                    | 1.40E+07 | 4.02E+06 | 2.58E+07 | 1.08E+07 | 8.90E+06 | 1.32E+06 | 1.87E+07 | 8.78E+06 |
| Th17                   | 2.06E+06 | 6.62E+05 | 5.89E+06 | 1.38E+06 | 2.42E+06 | 4.24E+05 | 3.05E+06 | 2.19E+06 |
| Tregs                  | 1.60E+07 | 2.64E+06 | 2.28E+07 | 4.34E+06 | 1.70E+07 | 2.85E+06 | 1.46E+07 | 3.69E+06 |
| CD8+ T cells           | 7.61E+07 | 1.33E+07 | 1.03E+08 | 1.85E+07 | 8.13E+07 | 2.83E+06 | 7.87E+07 | 2.77E+07 |
| B cells                | 5.20E+08 | 9.21E+07 | 6.17E+08 | 9.30E+07 | 5.60E+08 | 8.52E+07 | 4.90E+08 | 1.51E+08 |
| $\gamma\delta$ T cells | 1.62E+07 | 3.45E+06 | 1.94E+07 | 3.07E+06 | 1.64E+07 | 3.01E+06 | 1.53E+07 | 3.25E+06 |
| CD4+ NKT cells         | 1.35E+06 | 3.59E+05 | 1.80E+06 | 3.91E+05 | 1.35E+06 | 4.30E+05 | 1.25E+06 | 3.76E+05 |
| CD8+ NKT cells         | 6.42E+05 | 2.58E+05 | 8.60E+05 | 3.02E+05 | 6.08E+05 | 1.21E+05 | 5.13E+05 | 2.57E+05 |
| ILC1                   | 1.79E+05 | 1.23E+05 | 4.02E+05 | 3.49E+05 | 2.07E+05 | 3.06E+04 | 4.86E+05 | 4.21E+05 |
| NK                     | 5.82E+05 | 3.99E+05 | 1.13E+06 | 1.12E+06 | 5.54E+05 | 1.60E+05 | 2.64E+06 | 2.67E+06 |
| ILC2                   | 2.63E+06 | 8.57E+05 | 4.28E+06 | 1.67E+06 | 1.71E+06 | 6.44E+04 | 2.85E+06 | 1.36E+06 |
| ILC3                   | 9.33E+05 | 2.77E+05 | 1.47E+06 | 2.57E+05 | 8.74E+05 | 2.30E+05 | 7.83E+05 | 3.15E+05 |
| CD64+                  | 2.41E+06 | 1.28E+06 | 1.51E+06 | 8.15E+05 | 2.17E+06 | 1.11E+06 | 3.06E+06 | 1.73E+05 |
| cDC1                   | 3.75E+05 | 2.43E+05 | 3.74E+05 | 1.80E+05 | 8.18E+04 | 3.20E+04 | 3.80E+05 | 1.65E+05 |
| cDC2                   | 8.48E+06 | 2.05E+06 | 8.84E+06 | 2.47E+06 | 7.20E+06 | 2.69E+06 | 8.07E+06 | 2.21E+06 |
| CX3CR1+ DC             | 5.80E+06 | 1.95E+06 | 5.40E+06 | 1.15E+06 | 4.97E+06 | 1.39E+06 | 6.62E+06 | 5.64E+05 |
| Eosinophils            | 3.46E+06 | 1.26E+06 | 3.01E+06 | 5.70E+05 | 1.83E+06 | 1.15E+06 | 3.22E+06 | 4.27E+05 |
| Neutrophils            | 1.61E+07 | 6.08E+06 | 1.86E+07 | 6.42E+06 | 9.66E+06 | 3.83E+06 | 1.50E+07 | 3.40E+06 |

**Table S13. Spleen immune cell summary statistics in females fed HFHS.** VAM, vascular associated macrophage; Int, intermediate; DC, dendritic cell; cDC, conventional dendritic cell; NKT; natural killer T cell; Th, T helper cell; Treg, T regulatory cell; ILC, innate lymphoid cell; NK, natural killer cell.