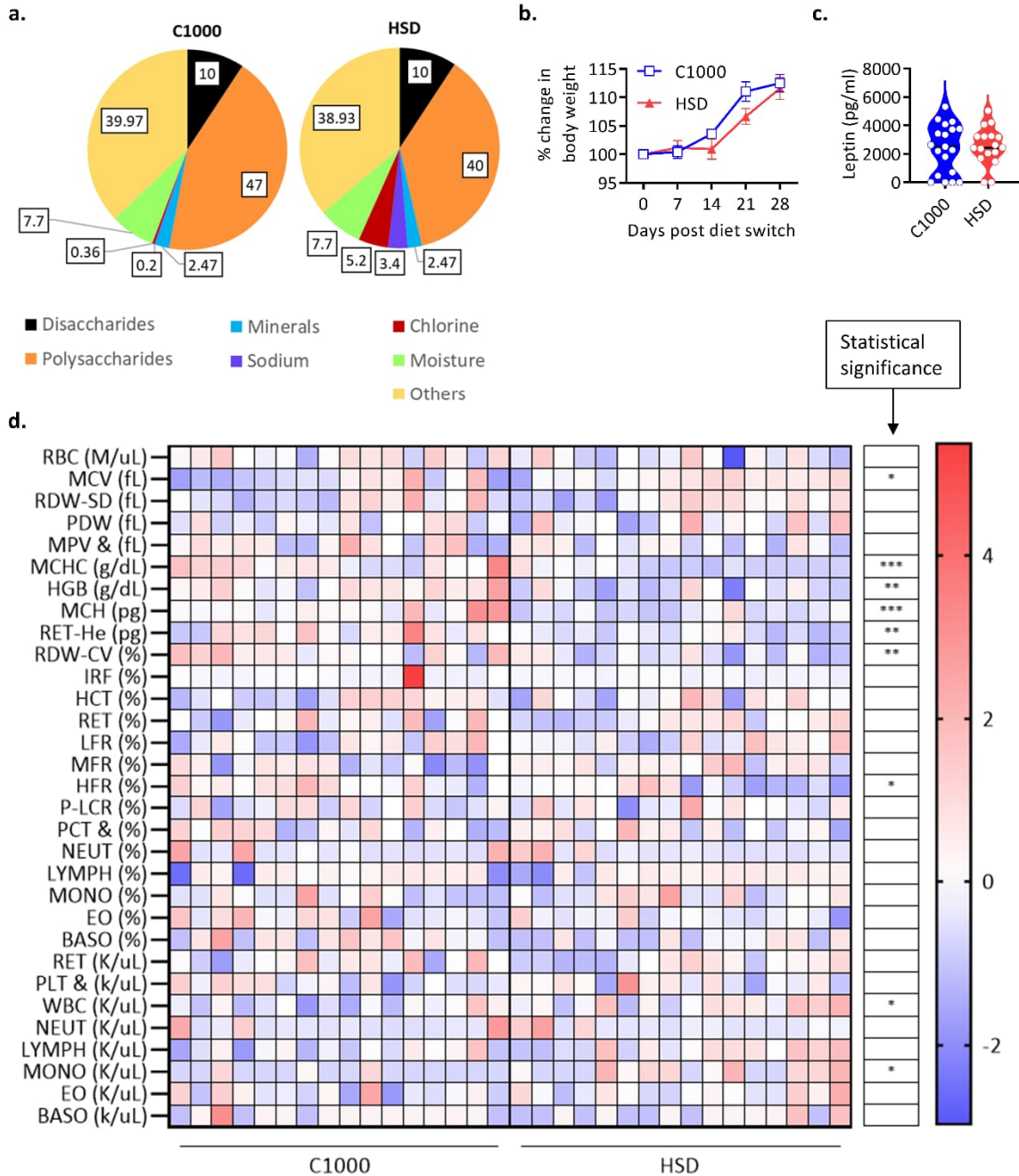
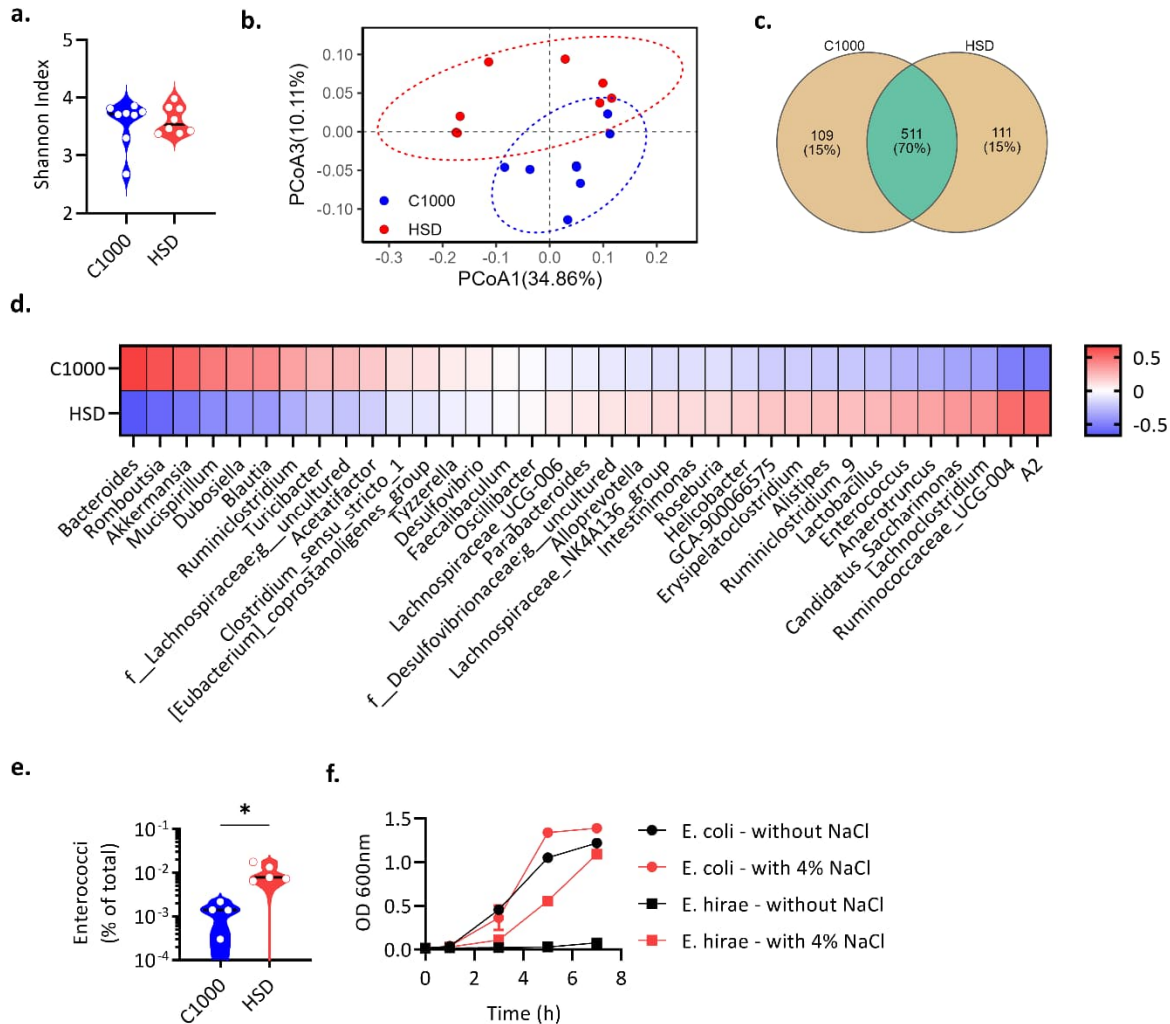


Supplementary figures

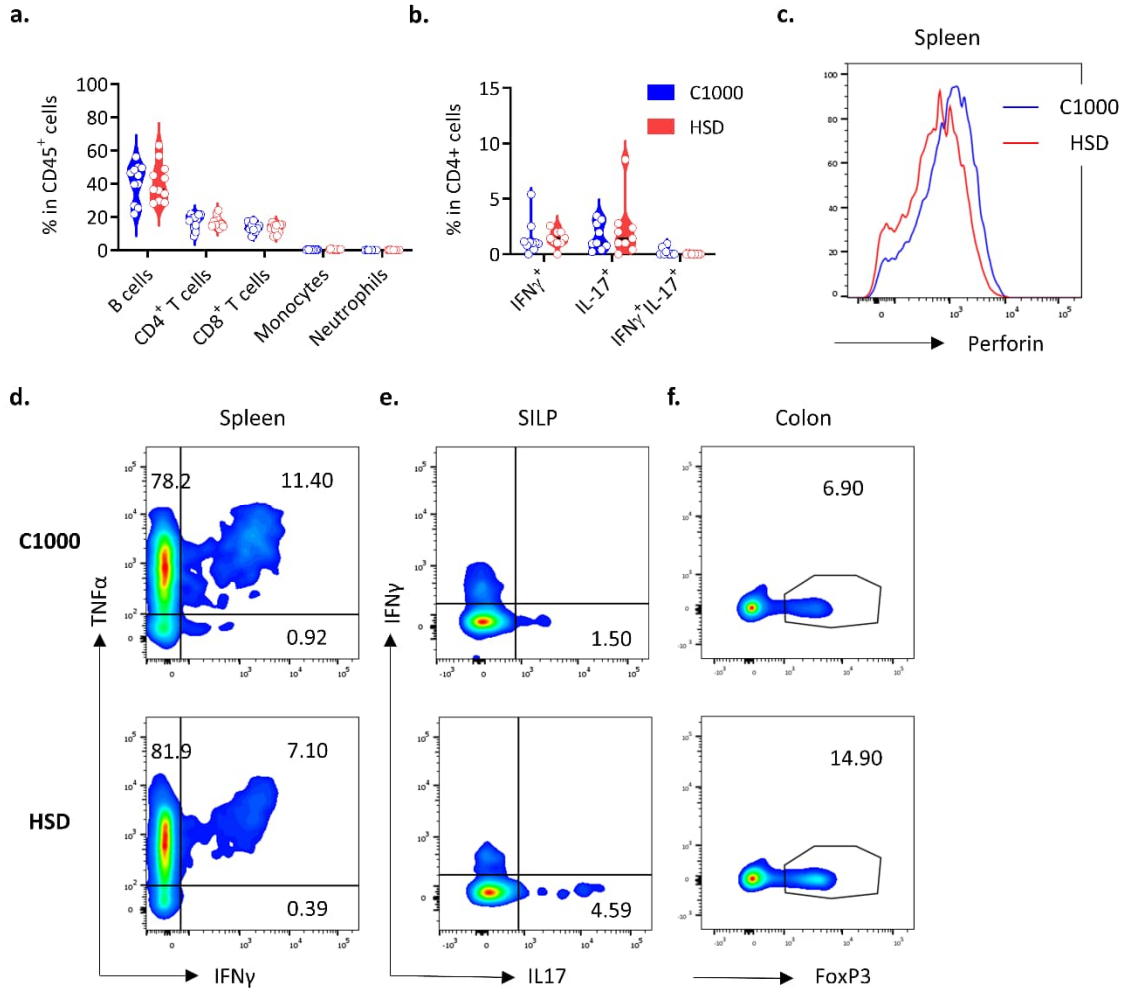


Supplementary figure 1: HSD is well tolerated by mice. (a) The chart shows the relative proportions of carbohydrates, minerals, sodium, chloride, and moisture in C1000 and HSD diets. (b) Bodyweight change in C57BL/6 mice after diet switch (C1000 n = 7, HSD n = 7). Data represented as mean \pm s.e.m. (c) Leptin levels in WT mice 4 weeks after diet switch (C1000 n=19, HSD n=17). Data from sera collected across 3 cohorts of mice is shown. Each circle represents an individual mouse. Data is represented as a distribution with the black line indicating the median.

(d) Heatmap of the complete blood count analysis on blood collected from C1000 (n = 16) and HSD (n = 16) fed C57BL/6 mice. *P<0.05, ** P<0.01, ***P<0.001 (Mann Whitney's U test). Values for all parameters were z-scored across the rows and represented in the heatmap. Data from sera collected across 2 cohorts of mice is shown. RBC, red blood cells; MCV, mean cell volume; RDW-SD, red blood cell distribution width-Standard deviation; PDW, platelet distribution width; MPV, mean platelet volume; MCHC, mean corpuscular hemoglobin concentration; HGB, hemoglobin; RET-He, reticulocyte Hb equivalent; RDW-CV, red blood cell distribution width-Coefficient of variation; IRF, immature reticulocyte fraction; HCT, hematocrit; RET, reticulocyte; LFR, reticulocytes with low fluorescence; MFR, reticulocytes with medium fluorescence; HFR, reticulocytes with high fluorescence; P-LCR, platelet-large cell ratio; PCT, procalcitonin; NEUT, neutrophils; LYMPH, lymphocytes; MONO, monocytes; EO, eosinophils; BASO, basophils; RET, reticulocytes; PLT, platelets; WBC, white blood cells; M/ul, millions per cubic milliliter; fl, femtoliters; g/dl, grams per deciliter; pg, picogram; K/ul thousands per cubic milliliter.



Supplementary figure 2: Gut microbiota alterations induced by HSD. (a-e) OSE mice were weaned onto C1000 (n = 8) or HSD (n = 8) and caecal content was collected 3-4 weeks after the diet switch. DNA was extracted and the 16s rRNA gene was sequenced. (a) Shannon diversity index of caecal microbiota from C1000 and HSD-fed mice. Each circle represents an individual mouse. Data is represented as a distribution with the black line indicating the median. (b) Venn diagram indicating the prevalence of the identified microbial OTUs in C1000 and HSD fed mice. (c) Principal coordinate analysis using Bray-Curtis dissimilarity matrix. (d) Heatmap showing the relative abundance of the most differentially abundant genera under C1000 and HSD (computed as a percentage of the total, then z-scored and represented). (e) 16s rRNA qPCR analysis of fecal DNA with Enterococci specific primers represented as a percentage of total microbiota (C1000 n = 4, HSD n = 5). OSE mice were weaned onto C1000 or HSD and fecal content was collected 3 – 4 weeks after the diet switch; *P = 0.015 (Mann Whitney’s U test). Each circle represents an individual mouse. One representative experiment out of 2 experiments performed is shown. Data is represented as a distribution with the black line indicating the median. (f) OD (600nm) at various time points during the culture of *Enterococcus hirae* and *Escherichia coli* in Brain Heart Infusion Broth (BHI) broth with or without 4 % NaCl. Data represented as mean ± s.e.m.



Supplementary figure 3: HSD alters cytokine-producing T cell populations. (a-f) C57BL/6 mice were weaned onto C1000 or HSD and lymph nodes were collected 3 – 4 weeks after the diet switch. (a) Frequencies (% of CD45⁺ cells) of various immune cell subsets in the lymph nodes of C57BL/6 mice fed with C1000 and HSD. n = 9 – 11 per group. Data from 3 individual experiments are pooled. (b) Frequencies (% of CD4⁺ T cells) of various cytokine-producing T cell subsets in the lymph nodes of C57BL/6 mice fed with C1000 and HSD. Data from 3 individual experiments are pooled. n = 9 – 11 per group. (c) Representative flow cytometry histograms showing the MFI of Perforins in splenic NK cells from C1000 and HSD fed mice. (d) Representative flow cytometry plots showing TNF α and IFN γ staining in splenic CD4⁺ T cells from C1000 and HSD-fed mice. (e) Representative flow cytometry plots showing IL17 staining in SILP CD4⁺ T cells from C1000 and HSD fed mice. (f) Representative flow cytometry plots showing Foxp3 staining in colonic CD4⁺ T cells from C1000 and HSD fed mice.