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Supplemental information

High-fat-diet-associated intestinal microbiota exacerbates psoriasis-like inflammation by enhancing systemic γδ T cell IL-17 production

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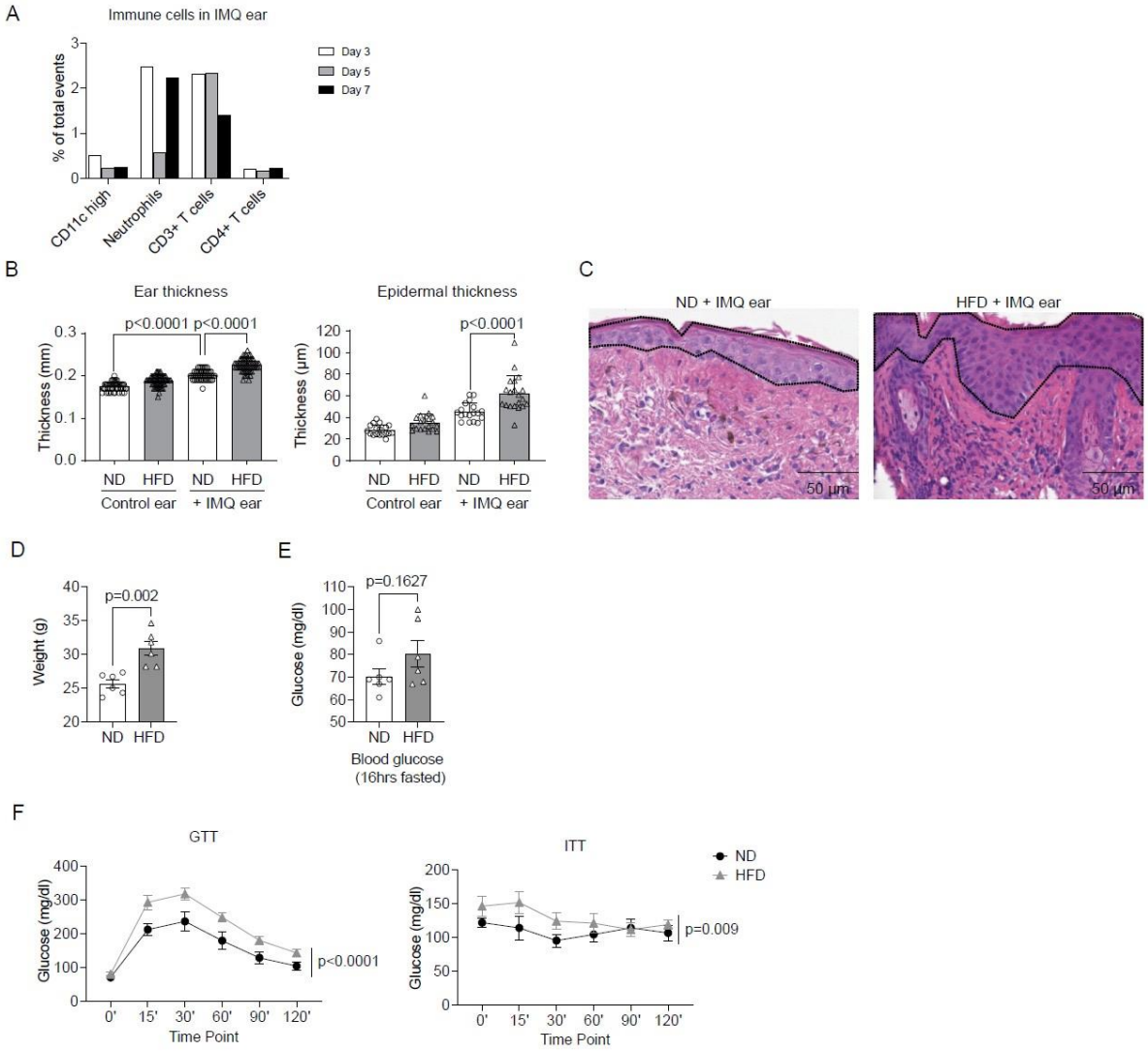

Figure S1. Immune cells infiltration in the ear, body weight and blood glucose level after HFD, related to Figure 1

(A) Immune cells in the inflammatory ear measured by flow cytometry on different time point post imiquimod (IMQ) treatment. Gating strategy is shown in supplementary figure 3 (B-D) C57BL/6 mice were initially fed with ND or HFD for 8 weeks. (B) Ear thickness measured by caliper (N = 40) and quantification of epidermal thickness in histology (N = 20) in C57BL/6 mice fed with normal diet (ND; 3.3% fat) or high-fat diet (HFD; 35.7% fat) for 8 weeks with or without IMQ ear treatment. (C) Representative H&E staining of sections of the skin of the ear; epidermis is highlighted by broken lines. (D) Body weight (E) Blood glucose level after 16 hours fasting. (F) Glucose tolerance test (GTT) and Insulin tolerance test (ITT). Number of mice per group for B-D (N = 6 per group). Bars show the mean ± SD. p-values were calculated by analysis of variance followed by Sidak's post-hoc tests for comparison of multiple groups or unpaired Student's t-test for D-E, and according to two-way ANOVA followed by Bonferroni post hoc test for F.

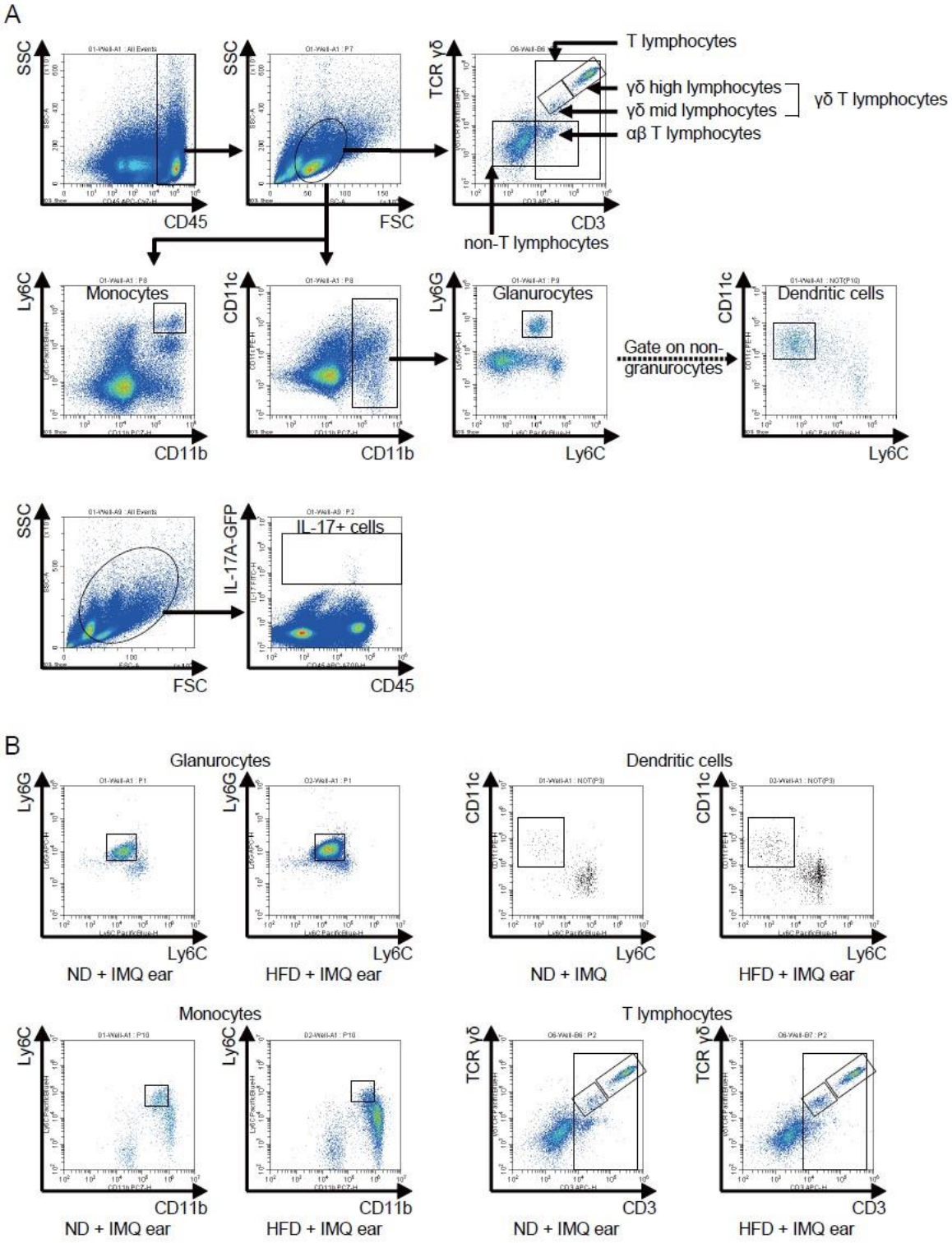

Figure S2. Gating strategies for immune cells in the affected skin, related to Figure 1

(A) Gating strategy for immune cell populations. (B) Representative dot plots of immune cells in the ear summarized in Figure 1C.

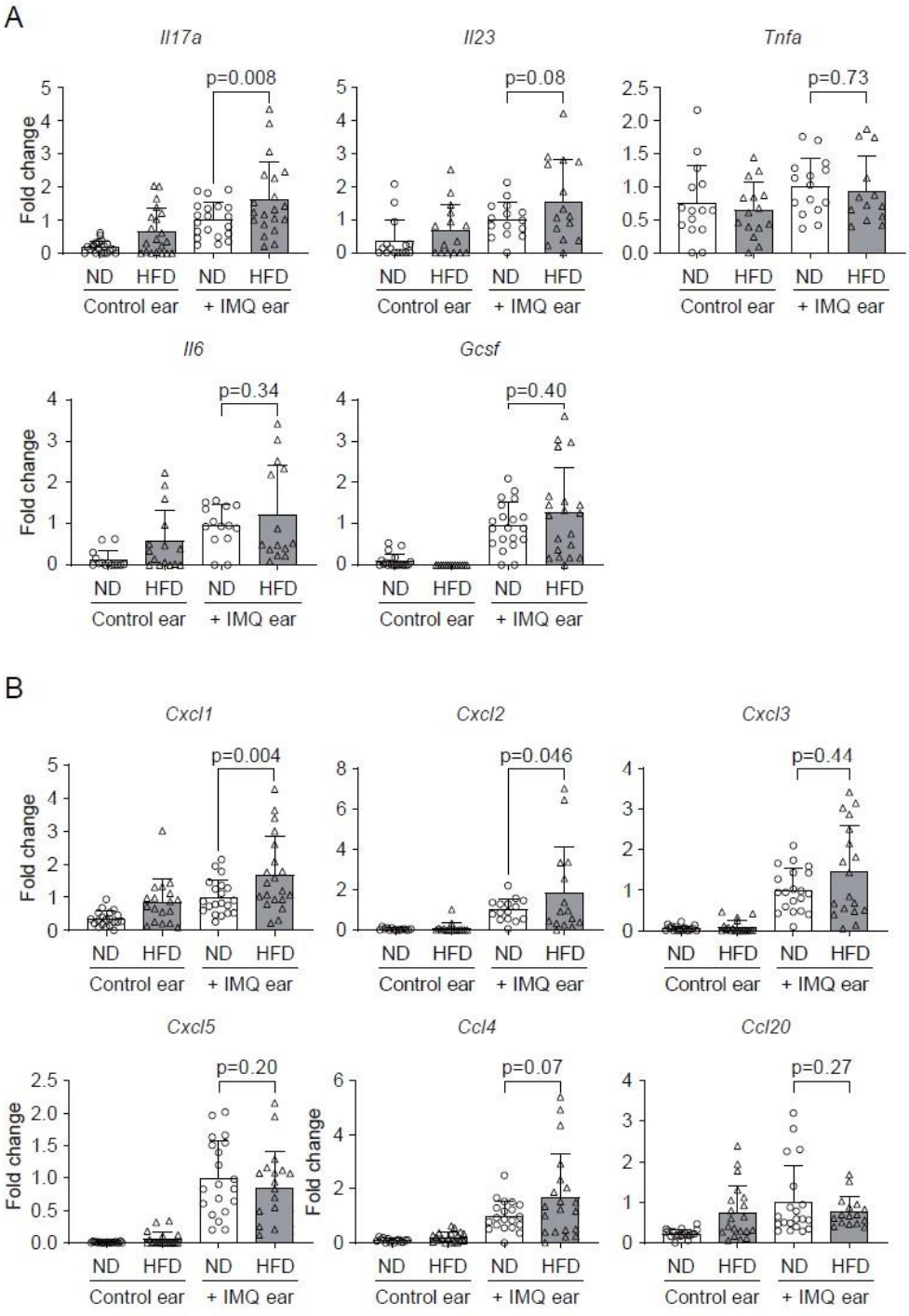

Figure S3. High fat diet induces *Il17, Il23* and chemokines expression in the skin, related to Figure 1

Real time PCR quantification of cytokines (A) and chemokines (B) mRNA levels in the affected (IMQ challenge for 3 days) and unchallenged skin of C57BL/6 mice fed with normal diet (ND; 3.3% fat) or high-fat diet (HFD; 35.7% fat). Bars show the mean ± SD, p-values were calculated by unpaired Student's t-test (N = 20).

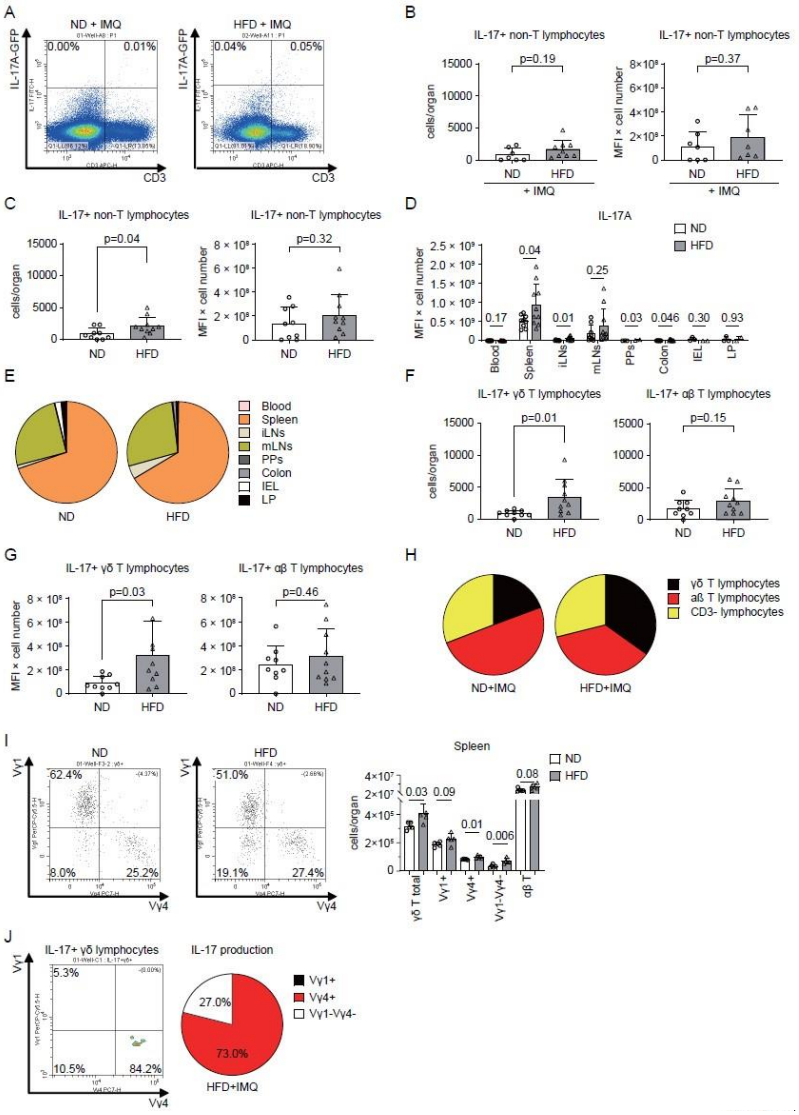

Figure S4. Induction of Vγ4+ cells by HFD, related to Figure 2

(A) Representative dot plot showing IL17+ γδ+ T lymphocytes in the spleen of IL-17 reporter mice fed with normal diet (ND; 3.3% fat) or high-fat diet (HFD; 35.7% fat) for 8 weeks and challenged with imiquimod (IMQ). (B) Number and IL-17 production of IL-17+ non-T lymphocytes in the spleen analysed by flow cytometry in IL-17 reporter mice fed with ND or HFD and challenged with IMQ (N = 7 - 9). (C) Number and IL-17 expression of IL-17+ non-T cells in the spleen in IL-17 reporter mice fed with ND or HFD without IMQ (N = 9 - 10). (D) IL-17 production from different organs and (E) contribution of organs to IL-17 production in IL-17 reporter mice fed with ND or HFD without IMQ (N = 9 - 10). (F) Number and (G) IL-17 production (MFI) of IL-17+ γδ and αβ T lymphocytes in the spleen (N = 9 - 10); (H) Contribution of IL-17 expressing cells to IL-17 production in the spleen. (I) Analysis of γδ T lymphocytes subset categorized by Vγ chain expression in the spleen of wild-type mice fed with ND or HFD without IMQ challenge. Plots show Vγ chain expressions of CD45+CD3+γδ TCR+ pre-gated cells. Number of lymphocyte subsets are shown in the bar graph (N = 5). (J) Plot shows the representative Vγ chain expression of CD45+CD3+γδTCR+IL-17+ pre-gated cells. The proportion of IL-17 production by γδ T lymphocytes subsets are shown in the circle graph. iLNs; inguinal lymph nodes. mLNs; mesenteric lymph nodes. PPs; Peyer's patches. IEL; intra-epithelial lymphocytes. LP; lamina propria cells. Bars show the mean ± SD, P-values were calculated by unpaired Student's t-test and are shown above each group.

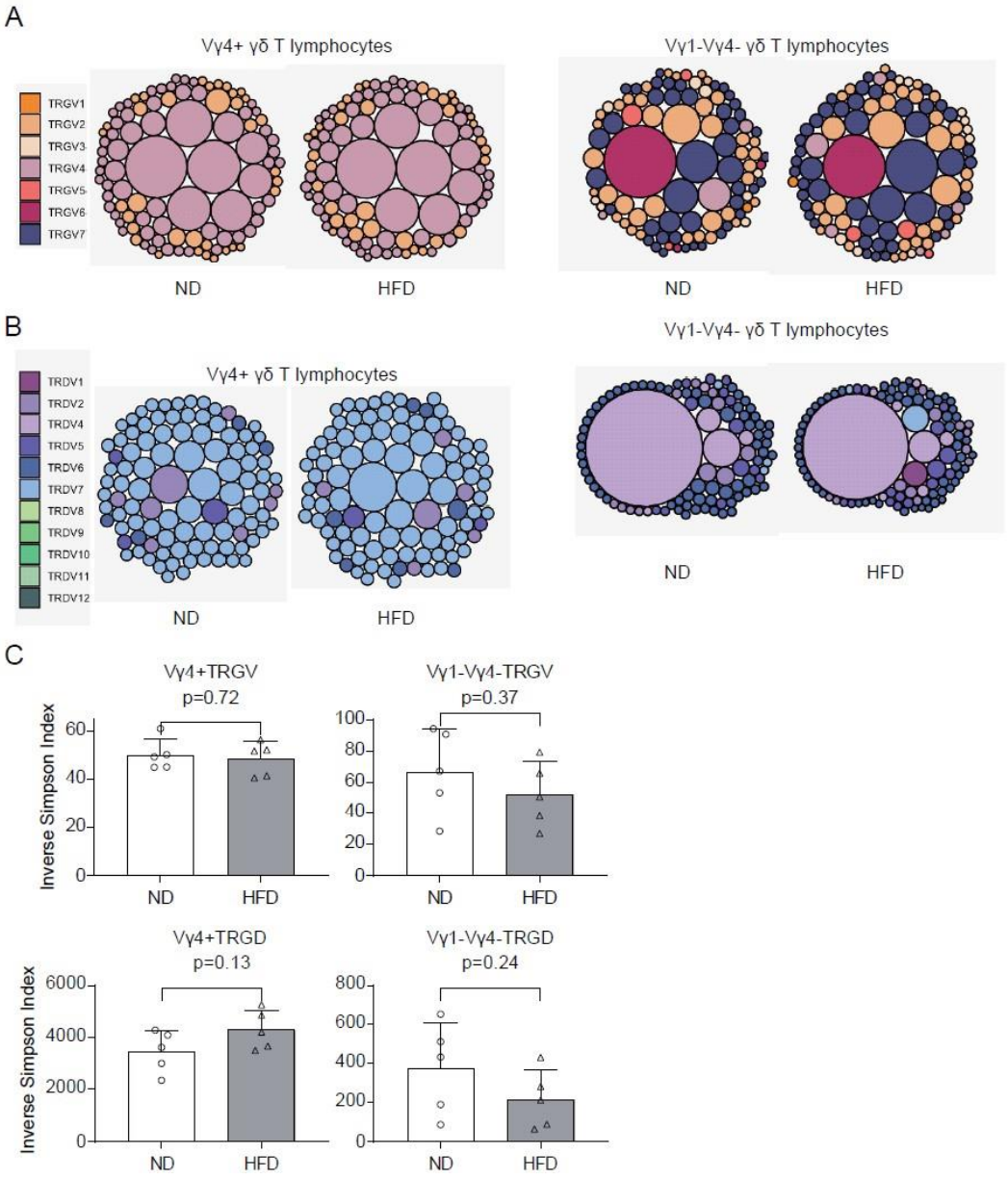

Figure S5. γδ TCR repertoire analysis, related to Figure 2

γ δ TCR+ lymphocytes according to their surface expression of Vγ chains sorted from the spleen of mice fed with normal diet (ND; 3.3% fat) or high-fat diet (HFD; 35.7% fat). CDR3 regions of TCRG and TCRD chains were amplified and sequenced. (A, B) Top 100 clonotypes based on AA sequence, colored according to TRG-V gene usage (A), or TRD-V gene usage (B) are shown. (C) The clonality of TRG and TRD repertoires is shown as Inverse Simpson index. P-values were calculated by Student's t-tests (N = 5).

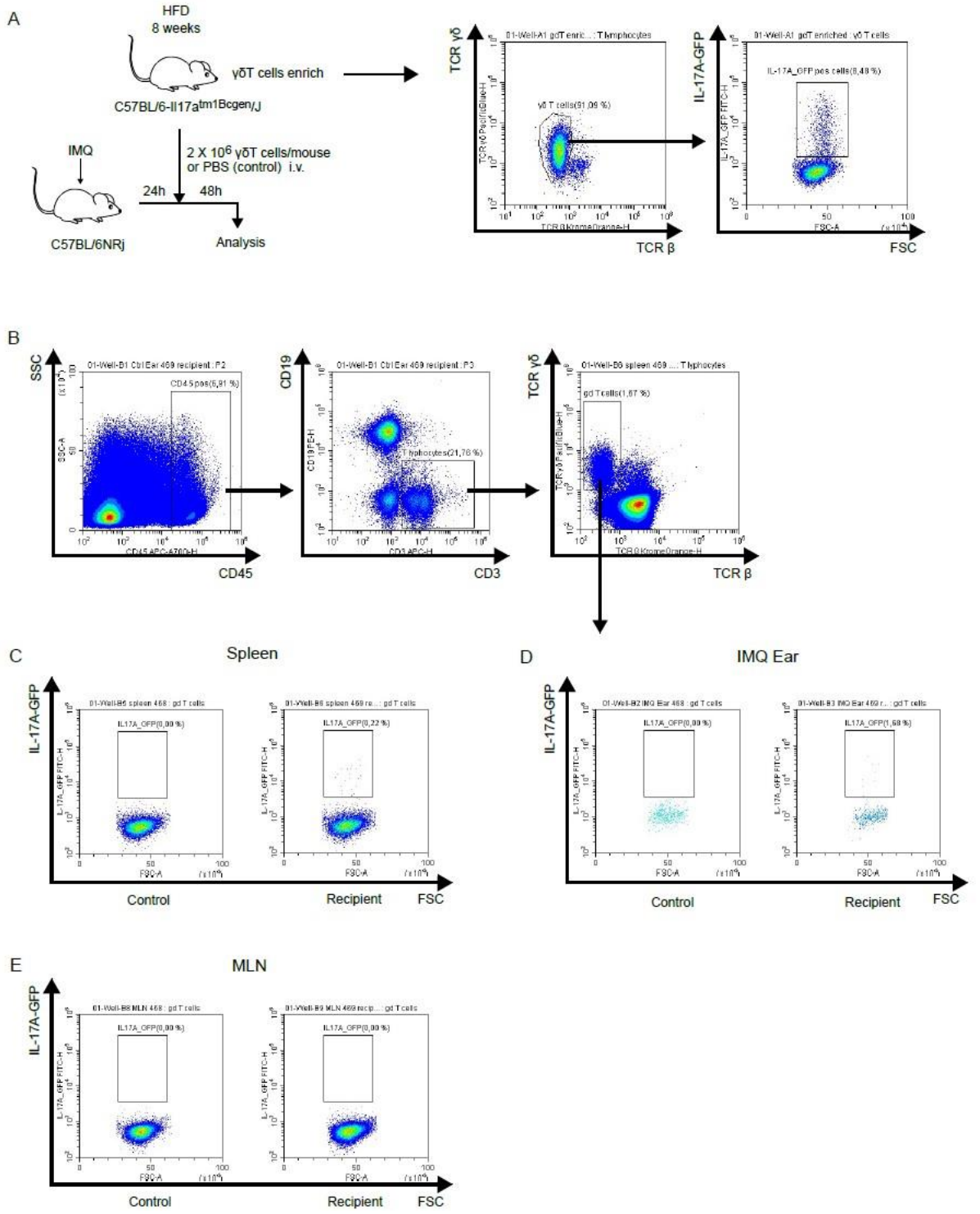

Figure S6. IL-17+ γδT cells from the spleen are recruited to the skin to increase dermal inflammation, related to Figure 2

(A) Scheme of adoptive transfer model. The flow cytometry graph shows the purity of γδT cell after enrichment and the proportion of IL-17+ γδT cells. (B) Gating strategy of IL-17+ γδT cells. (C-E) Proportion of IL-17+ γδT cell in spleen, imiquimod (IMQ) ear and mesenteric lymph nodes (mLNs) from mice with or without adoptive transfer.

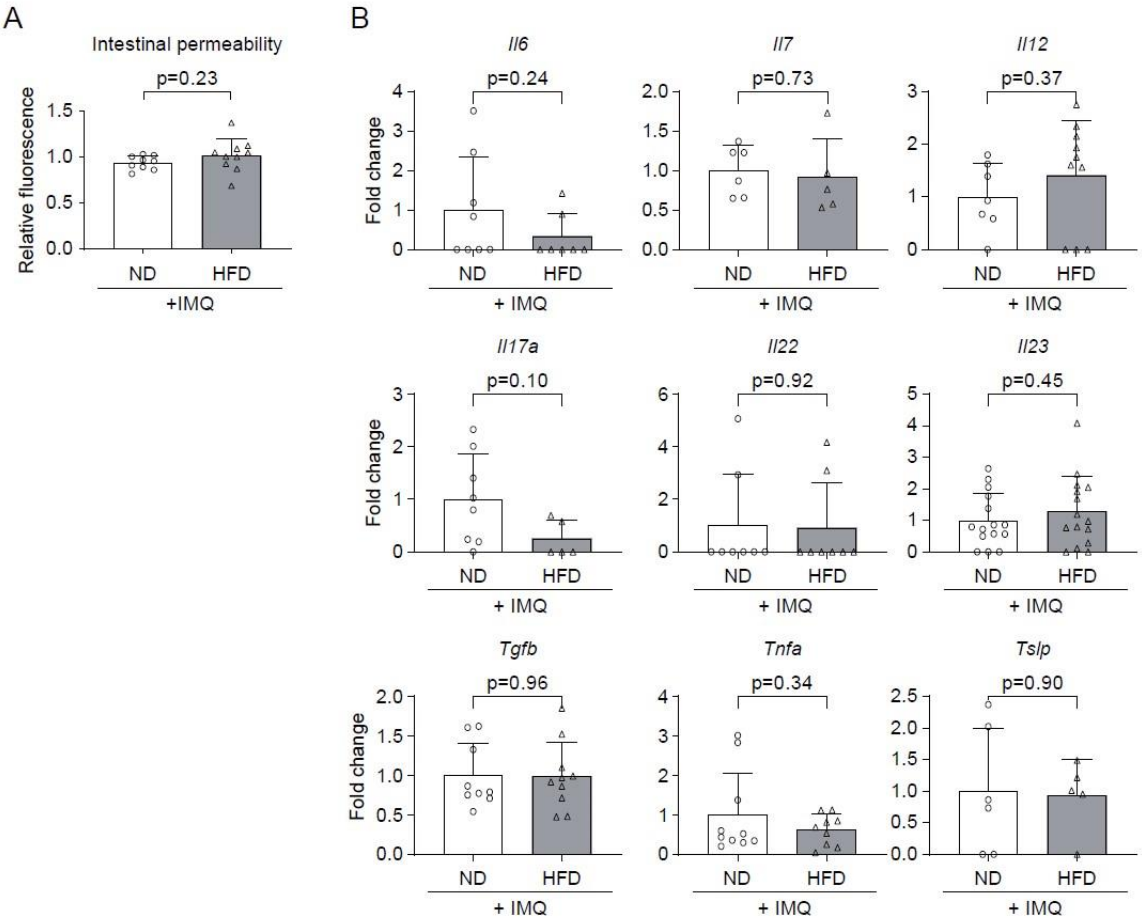

Figure S7. Barrier function and intestinal cytokines after dietary metabolic stress, related to Figure 3

C57BL/6 mice were fed with normal diet (ND; 3.3% fat) or high-fat diet (HFD; 35.7% fat) for 8 weeks and challenged with imiquimod (IMQ). (A) Mice received oral gavage of FITC-dextran (4 kDa) and their serum was collected after four hours starvation. Relative FITC fluorescence to ND group is shown (N = 5) (B) Real time PCR of cytokines in the small intestine (the first half of ileum) (N = 5). Bars show the mean ± SD, P-values were calculated by unpaired t-tests and are shown above each group.

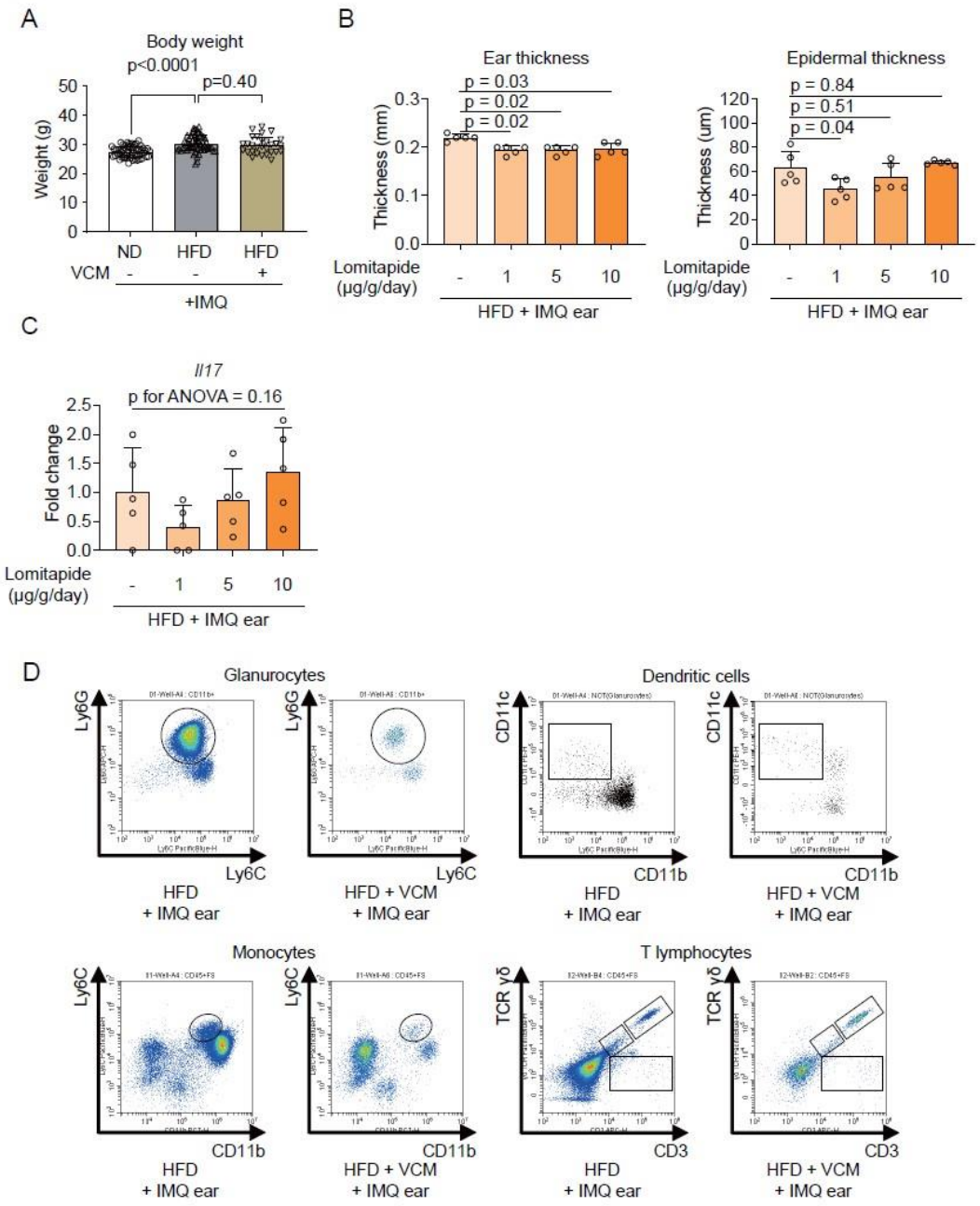

Figure S8. Body weight and adaptation of the microbiota changes after dietary metabolic stress, related to Figure 4

C57BL/6 mice were fed with normal diet (ND; 3.3% fat) or high-fat diet (HFD; 35.7% fat) for 8 weeks and challenged with imiquimod (IMQ). 0.5 mg/dl of vancomycin (VCM) was administrated as drinking water for the last two weeks. (A) Body weight. (HFD; N = 60, HFD + VCM; N = 30). (B, C) C57BL/6 mice were fed high-fat diet (HFD; 35.7% fat) for 8 weeks and were challenged with imiquimod (IMQ). Mice received lomitapide by oral gavage once daily for the last two weeks of the experiments. Group without lomitapide (described as " - " in the figures) received DPBS gavage instead. (B) Ear thickness measured by caliper and quantification of epidermal thickness in histopathology sections shown (N = 5); (C) Real time PCR of *Il17a* (N = 5). (D) Representative dot plots of flow cytometry analysis of immune cells in the skin from Figure 4D. Bars show the mean ± SD, p-values were calculated by analysis of variance followed by Sidak's post-hoc tests for comparison of groups indicated.

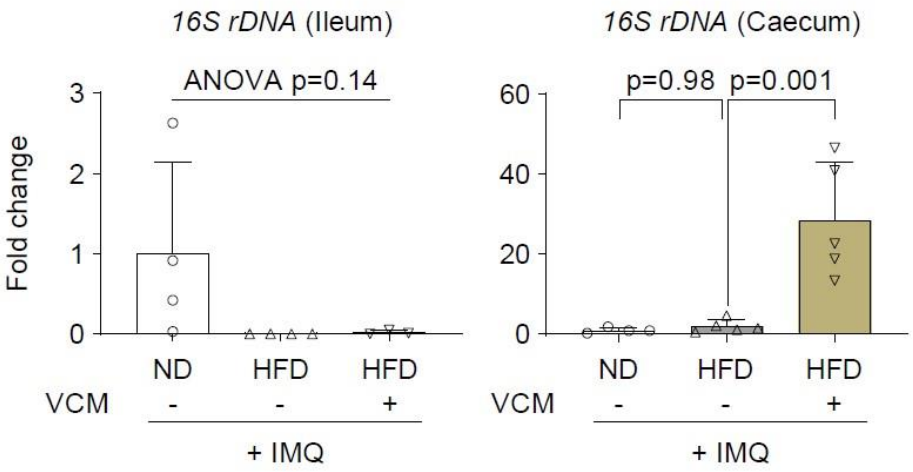

Figure S9. Real time PCR of intestinal bacteria, related to Figure 5

C57BL/6 mice were fed with normal diet (ND; 3.3% fat) or high-fat diet (HFD; 35.7% fat) for 8 weeks and were challenged with imiquimod (IMQ); real time PCR of bacterial 16S rDNA in the intestine. ND is the reference group (N = 5). Data are presented as the mean ± SD, p-values were calculated by analysis of variance followed by Sidak's post-hoc tests for comparison of groups indicated.

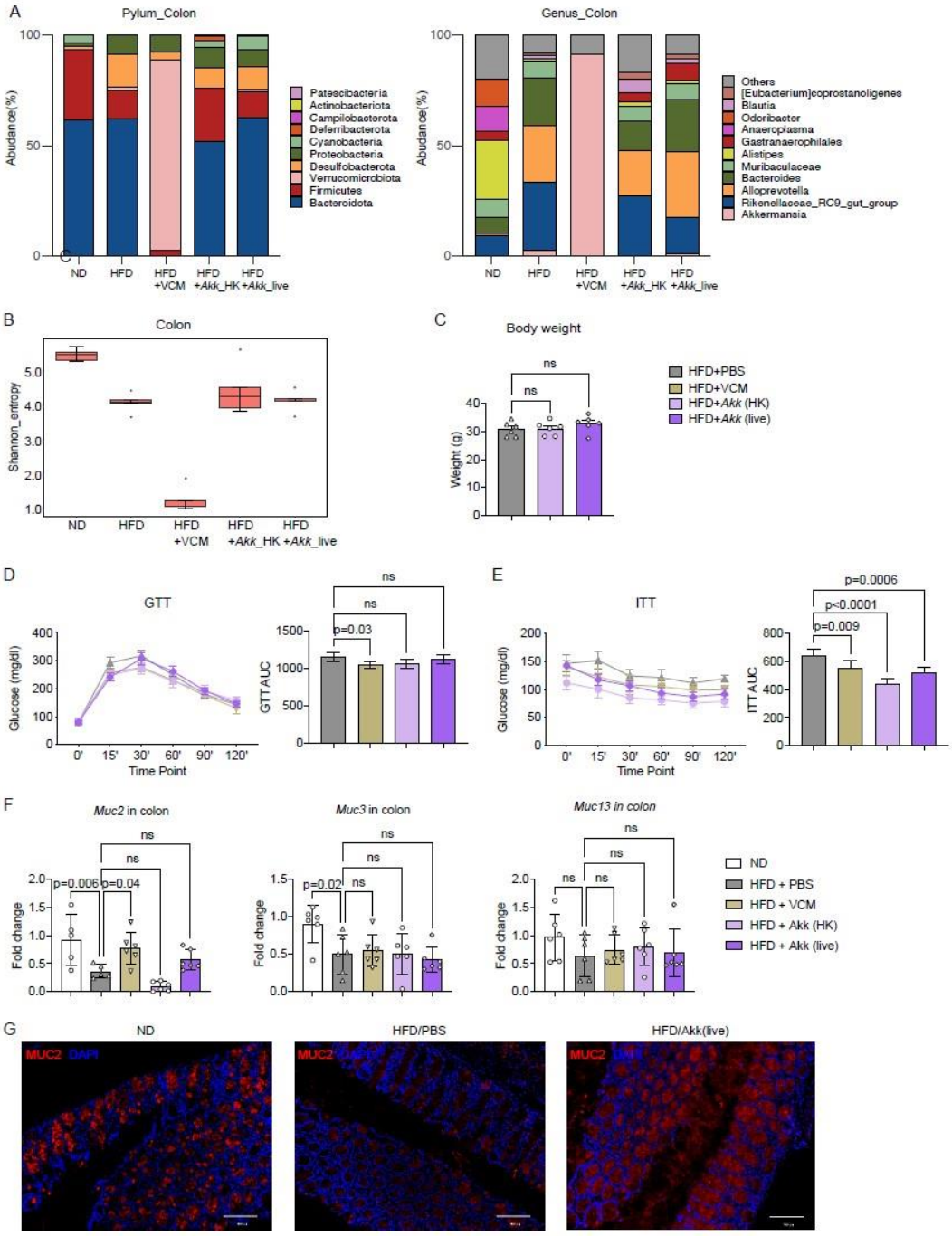

Figure S10. *Akkermansia muciniphila* effects on colon from HFD-fed mice, related to Figure 5

C57BL/6 mice were fed with normal diet (ND; 3.3% fat) or high-fat diet (HFD; 35.7% fat) for 8 weeks and challenged with imiquimod (IMQ). 0.5 mg/dl of vancomycin (VCM) was administrated as drinking water for the last two weeks. (A) Microbiome sequencing analysis of the bacterial 16S rDNA in the colon at the level of phylum and genus (N = 5). (B) Shannon diversity index indicated gut microbiota diversity. (C) Body weight. (N = 6 per group) (D, E) Glucose tolerance test (GTT) (D) and Insulin tolerance test (ITT) (E) were performed on different groups with measurement of blood glucose concentrations (Panel C-E: N = 6). (F) Real time qPCR of mucins mRNA expression in the colon (N = 5-6). (G) Representative pictures of Mucin-2 staining in the colon. Red; Mucin-2, Blue; DAPI. Data are presented as the mean ± SD, p values were calculated by analysis of variance followed by Sidak's post-hoc tests for comparison of groups indicated.

Table S1. Flow cytometry panels, related to STAR Methods (Flow cytometry section)

	Antigen	Color	Dilution
Panel 1	Ly6G	APC	1:1000
	CD45	APC-eFluor 780	1:1000
	Ly6C	BV421	1:1000
	CD11c	PE	1:1000
	CD11b	PE-Cy7	1:1000
	Fc Block		1:1000
Panel 2	CD4	PerCP-Cy5.5	1:1000
	CD3	APC	1:1000
	CD45	Alexa Fluor 700	1:1000
	CD8	APC-Cy7	1:1000
	TCR γδ	BV421	1:1000
	TCR β	BV510	1:1000
	CD19	PE	1:1000
	CD11b	PE-Cy7	1:1000
	Fc Block		1:1000
Panel 3	TCR Vγ1	PerCP-Cy5.5	1:1000
	CD3	APC	1:1000
	CD45	Alexa Fluor 700	1:1000
	CD11b	APC-Cy7	1:1000
	TCR γδ	BV421	1:1000
	TCR β	BV510	1:1000
	CD19	PE	1:1000
	TCR Vγ4	PE-Cy7	1:1000
	Fc Block		1:1000
Splenocytes sorting	TCR Vγ1	APC	1:250
	CD19	APC-Cy7	1:500
	TCR γδ	BV421	1:500
	TCR β	BV510	1:500
	CD3	PE	1:250
	TCR Vγ4	PE-Cy7	1:500
	Fc Block		1:500

Table S2. Gradient conditions for HILIC and RP chromatography, related to STAR Methods (Liquid Chromatography - Mass Spectrometry section)

HILIC, the eluent was A: water, 0.1% formic acid, 10 mM ammonium formate and B: acetonitrile 95%, 5% water, 0.1% formic acid, 10 mM ammonium formate. For RP, the eluent was A: water, 0.1% formic acid and B: methanol, 0.1% formic acid.

HILIC				**RP**			
t (min)	%A	%B	curve	t (min)	%A	%B	curve
0.0	0.0	100.0	5	0.0	99.5	0.5	5
2.0	0.0	100.0	5	11.0	2.0	98.0	1
14.0	70.0	30.0	6	15.0	2.0	98.0	5
16.5	70.0	30.0	5	15.5	99.5	0.5	5
17.5	0.0	100.0	5	20.0	99.5	0.5	-
30.0	0.0	100.0	-				

Table S3. List of primers used in this study, related to STAR Methods (Real-time PCR section)

Gene	Forward	Reverse
Il17a	5′ TAACTCCCTTGGCGCAAAAG 3′	5′ TCTTCATTGCGGTGGAGAGTC 3′
Il23	5′ ATCTTCAAAGGGGAGCCTGC 3′	5′ ATCCTCTGGCTGGAGGAGTT 3′
Tnfa	5′ CGGCATGGATCTCAAAGACAAC 3′	5′ AGATAGCAAATCGGCTGACG 3′
Il6	5′ TCCTTCCTACCCCAATTTCC 3′	5′ GCCACTCCTTCTGTGACTCC 3′
Gcsf	5′ TTGCTTCAGCTGGATGTTGC 3′	5′ TGGAAGGCAGAAGTGAAGGC 3′
Cxcl1	5′ TCCAGAGCTTGAAGGTGTTGCC 3′	5′ AACCAAGGGAGCTTCAGGGTCA 3′
Cxcl2	5′ CATCCAGAGCTTGAGTGTGACG 3′	5′ GGCTTCAGGGTCAAGGCAAACT 3′
Cxc3	5′ CCCCAGGCTTCAGATAATCA 3′	5′ TCTGATTTAGAATGCAGGTCCTT 3′
Cxcl5	5′ GAAAGCTAAGCGGAATGCAC 3′	5′ GGGACAATGGTTTCCCTTTT 3′
Ccl4	5′ TCCCACTTCCTGCTGTTTCTC 3′	5′ TCTGTCTGCCTCTTTTGGTCAG 3′
Ccl20	5′ GTGGGTTTCACAAGACAGATGG 3′	5′ AGGTTCACAGCCCTTTTCAC 3′
Muc2	5′ ATCCCGAAACCACGTCTGCA 3′	5′ CGCTTCAGGTGCACAGCAAA 3′
Muc3	5′ GACGGTGTTGAAGACCAAAAC 3′	5′ GGATGGGGAACTGGATCTTT 3′
Muc4	5′ AGATGGACGTCATTGTGCAG 3′	5′ GCAGTAATTCATGGGACAGGA 3′
Muc13	5′ GCAAGAGCAGCTACCATGAA 3′	5′ GAGGCCTGAGATGAACTACCC 3′

Table S4. Diet composition, related to STAR Methods (HFD-IMQ mouse model)

Component	Catalog No. of the manufacture	Diet 1 E15629	Diet 2 (ND) V1534	Diet 3 E15660	Diet 4 (HFD) E15772	Diet 5 E15149
fat	% of total Cal	6.0	9.0	24.0	54.0	94.0
		2.9	3.3	11.3	35.7	79.2
sugar		62.0	4.7	0.5	17.1	0.7
protein	%w/w	19.1	19.0	52.5	21.6	8
starch		0.1	36.5	0.9	0.9	0.6
others		15.9	36.5	34.8	24.7	11.5