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Supplemental information

GATA4 controls regionalization of tissue

immunity and commensal-driven immunopathology

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Figure S1. GATA4 controls regionalization of tissue metabolism and immunity

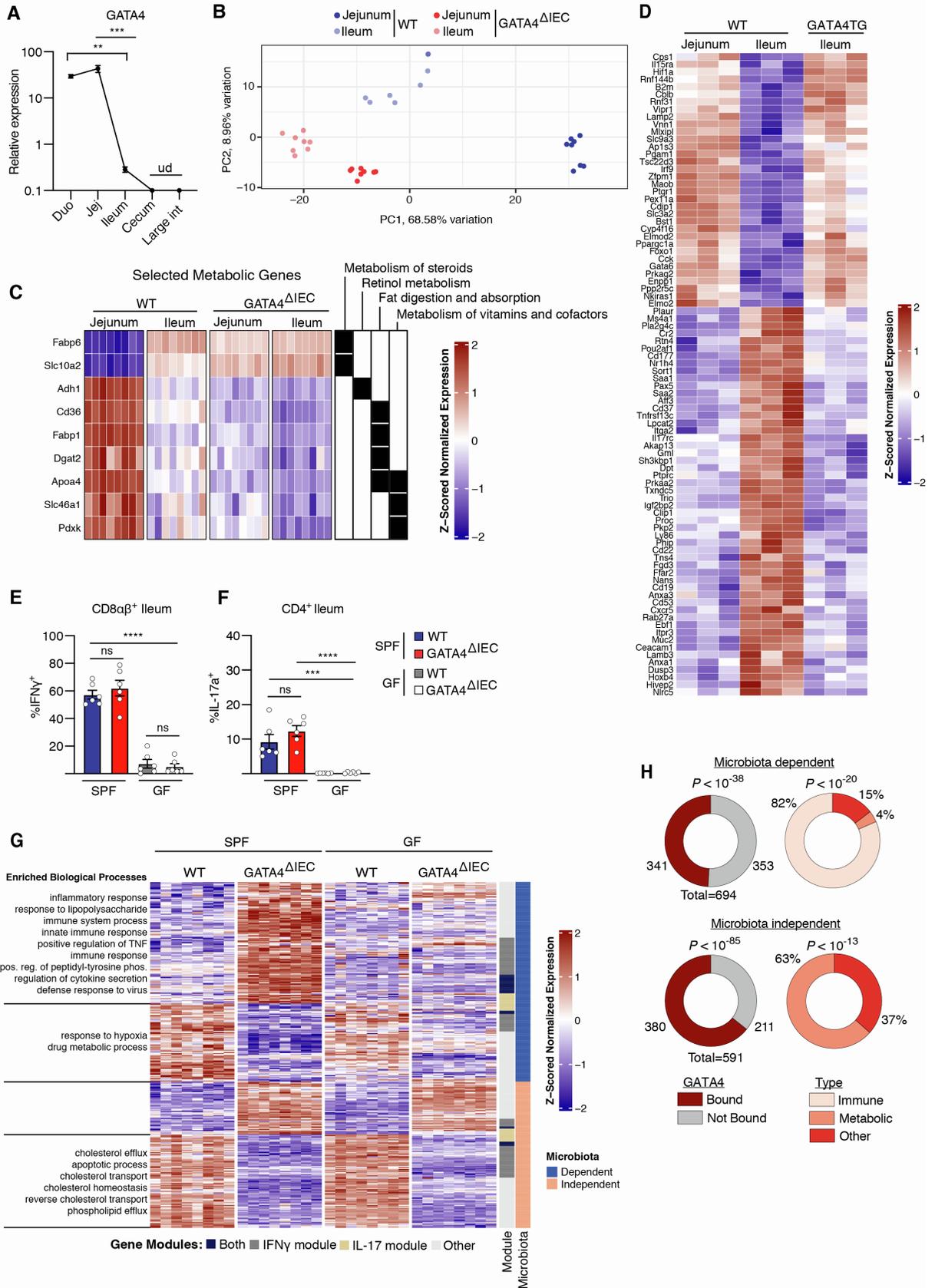


Figure S1. GATA4 controls regionalization of tissue metabolism and immunity

(A) Expression of GATA4, as measured by qPCR relative to *Gapdh*, in the tissue of all intestinal regions.

(B) Samples of epithelial cells from the jejunum and ileum of WT and GATA4^{ΔIEC} mice, plotted by the top two principal components of the RNA-seq based expression of the top 500 most variable genes (by standardized variance).

(C) Heatmap of z-scored expression, from RNA-seq of epithelial cells from the jejunum and ileum of WT and GATA4^{ΔIEC} mice, of selected genes in the following metabolic pathways: metabolism of steroids (Reactome), retinol metabolism (KEGG), fat digestion and absorption (KEGG), and metabolism of vitamins and cofactors (Reactome).

(D) Heatmap of region-specific immune genes, as measured by microarrays of mucosal scrapings from WT jejunum and ileum, and GATA4 transgenic (GATA4TG) ileum, from a published dataset⁹.

(E, F) Frequency of IFN γ ⁺ cells among CD8 $\alpha\beta$ ⁺ T cells (E), or of IL-17a⁺ cells among CD4⁺ T cells (F) in the ileum of SPF and GF WT and GATA4^{ΔIEC} mice. *N*= 6 mice/group.

(G) Heatmap of z-scored expression in tissue samples (columns) of all region-specific, GATA4-regulated genes (rows), annotated with enriched pathways (left) determined via DAVID^{55,56} (Table S1), microbiota dependence (right), and membership in IFN γ and IL-17 gene modules (right) from jejunum of SPF and GF WT and GATA4^{ΔIEC} mice.

(H) Left side: frequency of genes with promoters bound or not bound by GATA4, as determined by GATA4 ChIP-seq, among microbiota-dependent or microbiota-independent genes, as in E. While both sets of genes are significantly enriched in GATA4-bound promoters (341/694, $P < 10^{-38}$ and 380/591, $P < 10^{-85}$, respectively; Fisher's exact test), microbiota-independent genes have a significantly higher enrichment (odds ratio 1.86, $P < 10^{-7}$; Fisher's exact test, Table S1). Right side: percentage of genes enriched in different types of biological processes (immune, metabolic, other), in microbiota-dependent and microbiota-independent genes (Table S1). Microbiota-dependent genes show an enrichment in immune-related processes (82%, $P < 10^{-20}$), and microbiota-independent genes show an enrichment in metabolic related processes (63%, $P < 10^{-13}$), Fisher's exact test.

All data in this figure are pooled from at least two-independent experiments and represented as mean or mean \pm SEM. **** $P < 0.0001$, *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, ANOVA with Tukey multiple comparison test *N*= 6 mice/group. T-test (A), ANOVA with Tukey multiple comparison test (E,F)

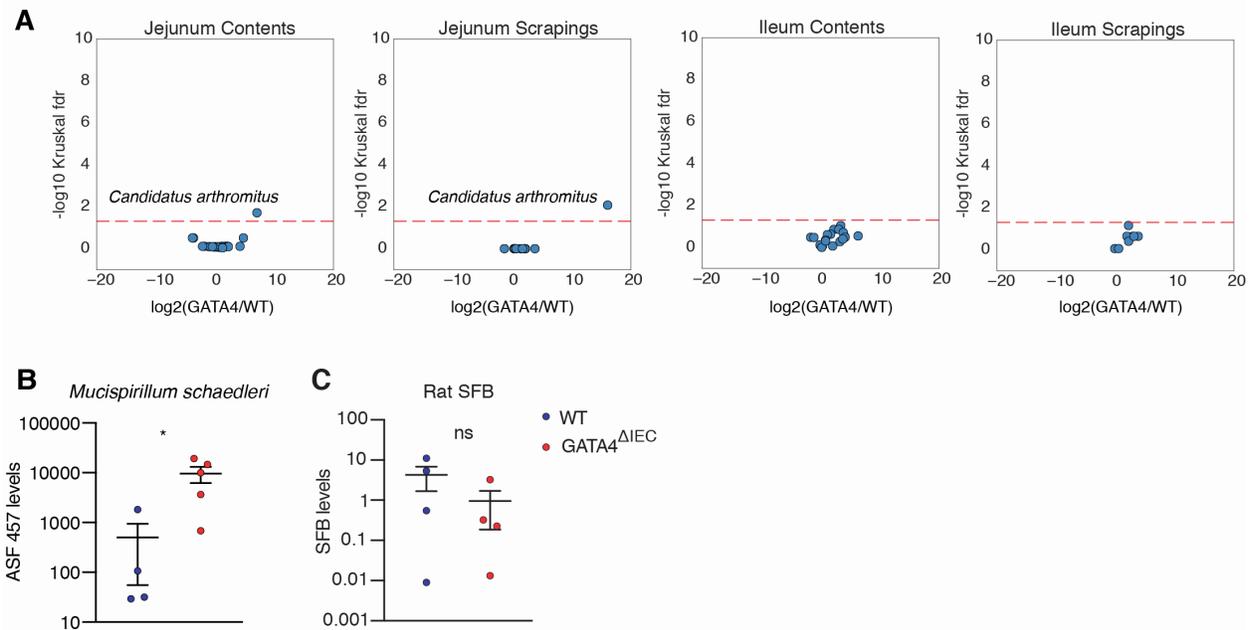


Figure S2. GATA4 controls the colonization of segmented filamentous bacteria.

(A) Relative frequencies (x axis, \log_2 fold change) in $\text{GATA4}^{\Delta\text{IEC}}$ versus WT mice of different bacteria taxa (dots), and their statistical significance (y axis, $-\log_{10}$ of the FDR-adjusted P value), based on 16S rRNA sequencing of the luminal contents and mucosal scrapings of the jejunum and ileum of WT and $\text{GATA4}^{\Delta\text{IEC}}$ mice. SFB are classified as *Candidatus arthromitus*.

(B) *Mucispirillum schaedleri* load, as measured by qPCR relative to host DNA in the jejunum of ASF colonized WT and $\text{GATA4}^{\Delta\text{IEC}}$ mice. * $P < 0.05$, Mann-Whitney. $N = 4-5$ mice/group.

(C) Rat SFB load, as measured by qPCR relative to host DNA in the jejunum of monocolonized WT and $\text{GATA4}^{\Delta\text{IEC}}$ mice. $N = 4$ mice/group.

All data in this figure are pooled from at least two-independent experiments and represented as mean or mean \pm SEM.

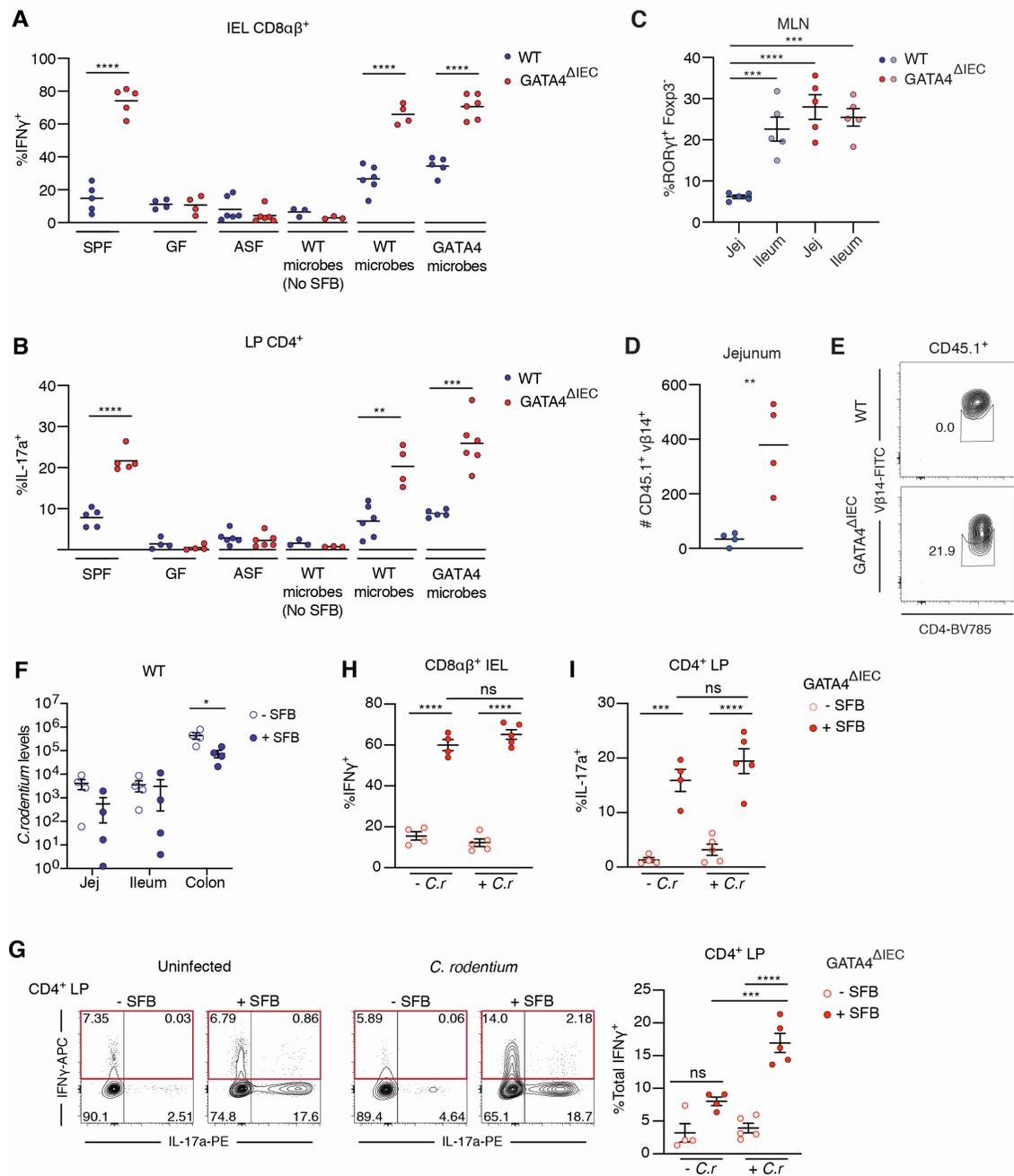


Figure S3. GATA4 controls the regionalization of tissue immune responses through commensal and pathogenic bacteria.

(A, B) Frequencies of IFN γ ⁺ cells among CD8 $\alpha\beta$ ⁺ T cells (A), and of IL-17a⁺ cells among CD4⁺ T cells (B) in the jejunum of WT and GATA4 Δ IEC mice with different microbiota (x axis), *i.e.*, SPF, GF, ASF, WT microbiota (no SFB), WT microbiota (with SFB), or GATA4 Δ IEC microbiota (with SFB). *N* = 3-6 mice/group.

(C) Frequency of ROR γ t⁺ FOXP3⁻ cells among transferred (CD45.1⁺ CD4⁺ V β 14⁺ 7B8⁺) SFB specific T cells in the MLNs draining the jejunum and ileum of WT and GATA4 ^{Δ IEC} mice three days after transfer. *N*= 5 mice/group.

(D) Number of transferred T cells (as in C) in the jejunum LP 9 days after transfer. *N*= 4 mice/group.

(E) Representative plot showing frequency of downregulation of v β 14 TCR among transferred CD4⁺ CD45.1⁺ 7B8⁺ T cells in the jejunum as in (D).

(F) *C. rodentium* load, measured by qPCR relative to host DNA, in distinct intestinal segments in SFB free or SFB colonized WT mice. *N*= 4 mice/group.

(G) Representative (left) plots and summarized (right) of IFN γ ⁺ and IL-17a⁺ CD4⁺ LP T cells from the jejunum of GATA4 ^{Δ IEC} mice that are colonized with JAX or JAX + SFB, and either uninfected (- *C.r*) or infected (+ *C.r*) with *C. rodentium*. Red box indicates total IFN γ ⁺ CD4⁺ T cells which are summarized (right). Mice were analyzed 5 days after infection. *N*= 4-5 mice/group.

(H, I) Frequencies of IFN γ ⁺ CD8 α β ⁺ IEL (H), or IL17a⁺ CD4⁺ LP (I), from the jejunum of GATA4 ^{Δ IEC} mice as in (G). *N*= 4-5 mice/group.

All data in this figure are pooled from at least two-independent experiments and represented as mean or mean \pm SEM. *****P*<0.0001, *** *P*<0.001, ** *P*<0.01, . * *P*<0.05, t-test (A, B, D), ANOVA with Tukey multiple comparison test (C, G, H, I), Mann-Whitney test (F)

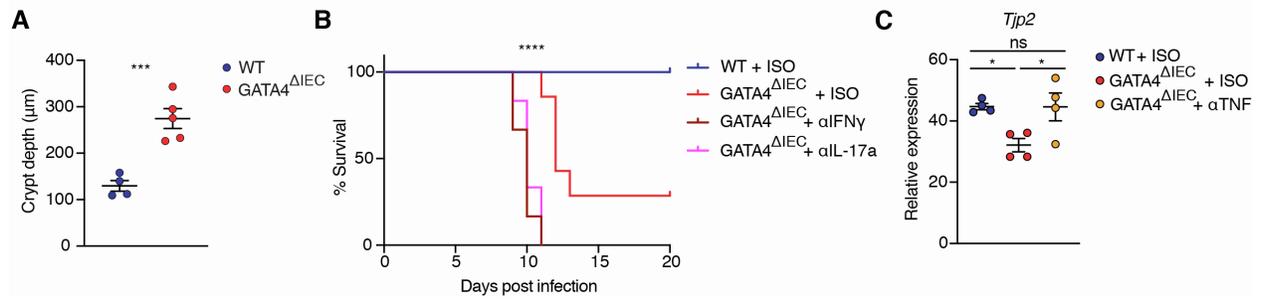


Figure S4. Presence of SFB increases host susceptibility to *C. rodentium* infection in GATA4-deficient mice.

(A) Colonic crypt depth (from Figure 4A) measured in μm of *C. rodentium* infected WT and GATA4 ΔIEC mice 10 days after infection. $N=4-5$ mice/group. *** $P<0.001$, t-test.

(B) Percent survival of WT and GATA4 ΔIEC isotype treated, $\alpha\text{IFN}\gamma$, or $\alpha\text{IL-17a}$ treated mice 0–20 dayspost *C. rodentium* infection. $N= 6-7$ mice/ group.

(C) Relative expression as measured by qPCR of *Tjp2* to GAPDH in the jejunum tissue of WT and GATA4 ΔIEC isotype treated, or $\alpha\text{TNF}\alpha$ treated 5 days after infection. $N= 4$ mice/group

All data in this figure are pooled from at least two-independent experiments and represented as mean or mean \pm SEM. **** $P<0.0001$, *** $P<0.001$, ** $P<0.01$, . * $P<0.05$, t-test (A), Mantel-Cox test (B), ANOVA with Tukey multiple comparison test (C).

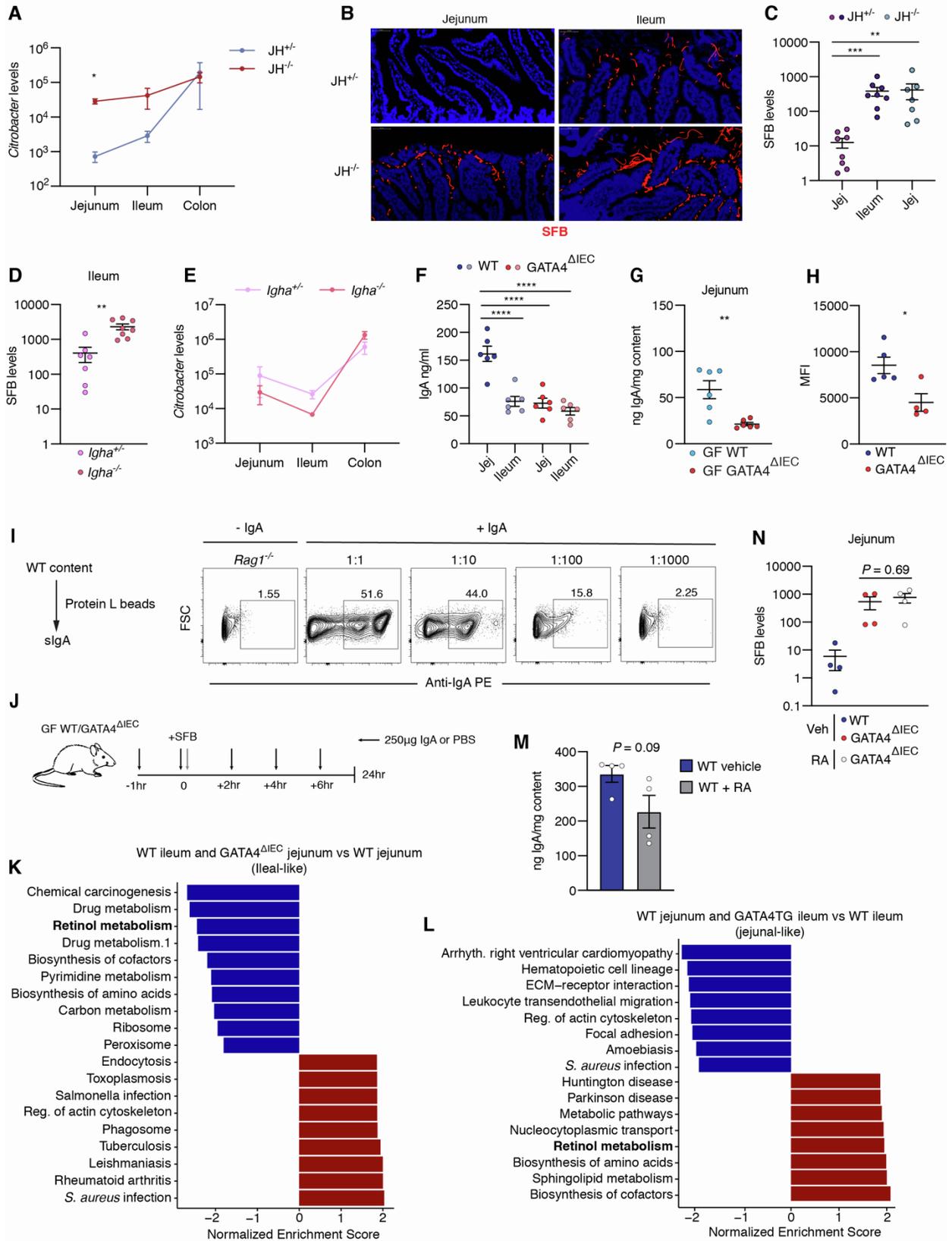


Figure S5. GATA4 regulates regionalization of retinol metabolism and B cell responses.

- (A) Bacterial loads of *C. rodentium*, measured by qPCR in distinct intestinal segments of GF JH^{+/-} or JH deficient mice. *N*= 4-5 mice/group.
- (B) FISH staining of SFB (Cy5) in jejunal and ileal tissue of monocolonized B-cell deficient (JH) and littermate control (Jh^{+/-}) mice and counterstained with DAPI.
- (C) SFB load, as measured by qPCR, in mucosal scrapings of B-cell deficient and control mice from (B). *N*= 7-8 mice/group.
- (D) SFB load, as measured by qPCR, in mucosal scrapings from the ileum of control (*Igha*^{+/-} mice and IgA deficient (*Igha*^{-/-}) mice. *N*= 7-8 mice/group.
- (E) Bacterial loads of *C. rodentium*, measured by qPCR, in distinct intestinal segments of GF *Igha*^{+/-} or *Igha*^{-/-} mice. *N*= 4-5 mice/group.
- (F) Concentration of IgA, as measured by ELISA, in culture supernatant of tissue explants from the jejunum and ileum of WT and GATA4^{ΔIEC} mice. *N*= 6 mice/group.
- (G) Concentration of IgA, as measured by ELISA, in jejunal content of GF WT and GATA4^{ΔIEC} mice. ** *P*<0.01, t-test.
- (H) Mean fluorescence intensity, of IgA⁺ bacterial coating (as in Fig. 5E). *N*= 4-5 mice/group.
- (I) Schema (left) for isolation of sIgA from luminal contents of WT mice, and FACS plots (right) showing the frequency of IgA-coated bacteria after staining feces of *Rag1*^{-/-} mice with isolated sIgA.
- (J) Experimental schema of IgA gavage and SFB colonization experiment in GF WT and GATA4^{ΔIEC} mice.
- (K, L) Pathways in the KEGG database that are significantly enriched in epithelial, region-specific, GATA4-regulated genes, and their respective normalized enrichment scores (NES, x axis) (Table S1, FDR-adjusted *P*< 0.01, |NES| > 1.75; fgsea), in comparing WT jejunum versus WT ileum and GATA4^{ΔIE} jejunum (K), or WT ileum versus WT jejunum and GATA4^{TG} ileum (L)⁹.
- (M) Total IgA in the jejunal contents of WT vehicle-treated and WT RA-treated mice after 14 days. *P*= 0.09, *N* = 4 mice/group
- (N) SFB loads, as measured by qPCR, in jejunal mucosal scrapings in WT, GATA4^{ΔIEC} vehicle-treated, and GATA4^{ΔIEC} RA-treated mice. *P*= 0.69, t- test, comparing GATA4^{ΔIEC} vehicle-treated and GATA4^{ΔIEC} RA-treated mice. *N* = 4 mice/group
- All data in this figure are pooled from at least two-independent experiments and represented as mean or mean ± SEM. *****P*<0.0001, *** *P*<0.001, ** *P*<0.01, * *P*<0.05, ns *P*>0.05, Mann-Whitney test (A, E), Kruskal-Wallis with Dunn multiple comparison test (C), Mann-Whitney test (D), ANOVA with Tukey multiple comparison test (F), t-test (G, H, M, N)

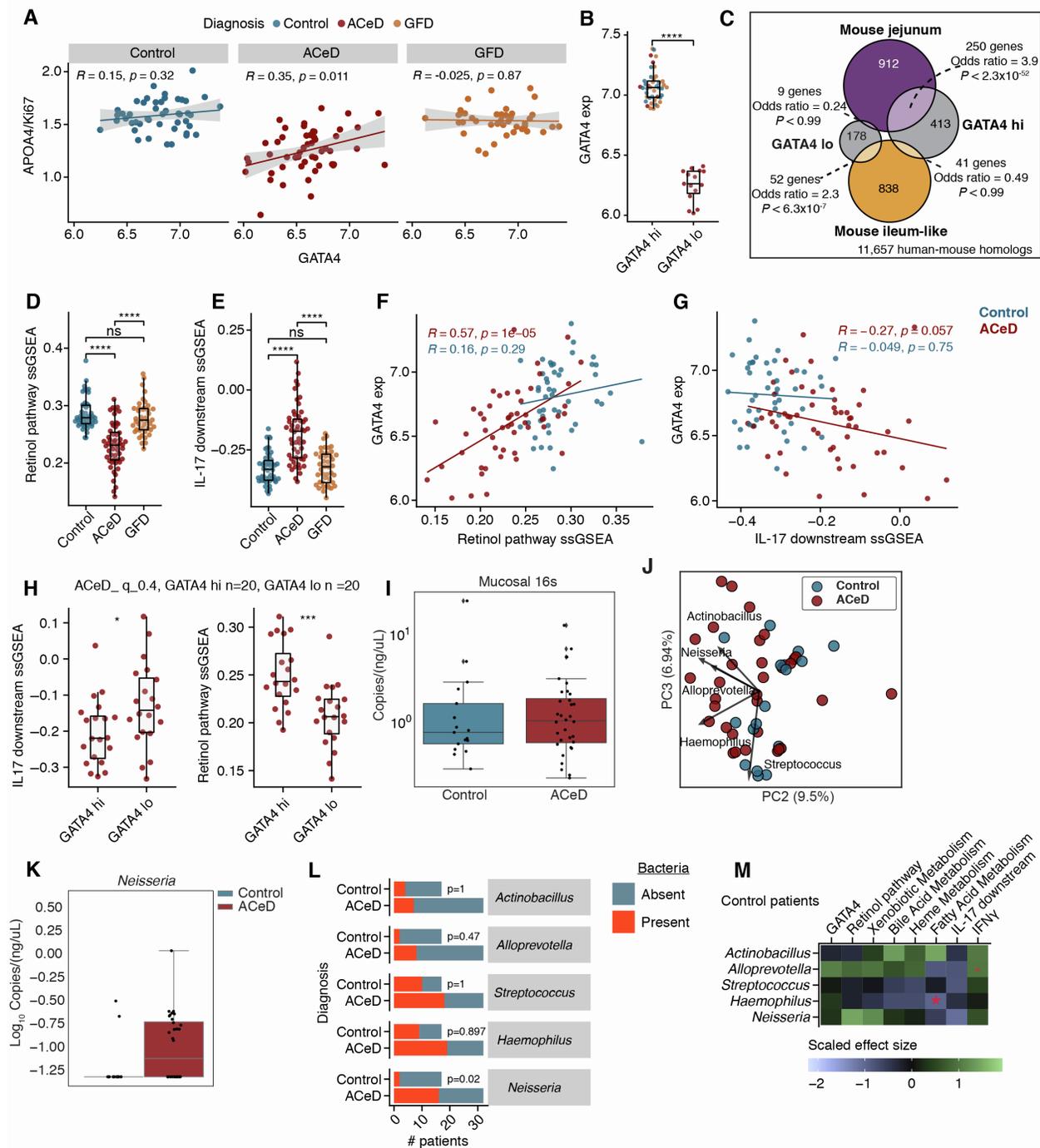


Figure S6. Loss of GATA4 is associated with lipid metabolic defects, mucosa-associated bacteria, and increased IL-17 signaling in celiac disease patients.

(A) Scatter plot of the ratio of *APOA4* to *KI67* expression, as a proxy for villi-crypt ratio, by disease group. Figure is annotated with spearman correlation coefficient (R) and p-value (p).

(B) *Gata4* expression in GATA4-hi and GATA4-lo individuals from control (blue), ACeD (red), and GFD (orange) patients. GATA4-lo: 15 ACeD patients; GATA4-hi: 18 control, 6 ACeD, and 18 GFD.

(C) Euler diagram represents the overlaps, in a common universe of 11,657 mouse-human transcriptional homologs (Table S3), of 4 gene sets: GATA4-regulated jejunum-specific (purple) and ileum-like-specific (yellow) genes, and GATA4-hi specific (right, gray) and GATA4-lo ACeD specific (left, gray) genes. Enrichment significance as annotated.

(D) Retinol pathway ssGSEA scores in individuals from control, ACeD, or GFD patient groups.

(E) IL-17 downstream signaling pathway ssGSEA scores, analogous to C.

(F) Scatter plot of GATA4 normalized expression and ssGSEA scores for the retinol metabolism pathway in control and ACeD patient groups. Annotated with Pearson correlation coefficient and p-value.

(G) Scatter plot of GATA4 normalized expression and ssGSEA scores for the IL-17 downstream signaling pathway, analogous to D.

(H) Single-sample gene set enrichment analysis (ssGSEA) scores for IL-17 downstream signaling (left), the retinol metabolism (right) in ACeD patients with GATA4 expression above (GATA4 hi) and below (GATA4 lo) the 40th percentile. The 40th percentile threshold is used in the analysis of ACeD patients (compared with the 30th percentile in Figure 6E) to ensure enough data points are included.

(I) Box plots of absolute abundances of mucosal 16S in biopsies from control or ACeD patients. Kruskal-Wallis $P < 0.64$.

(J) Principal component analysis (PCA) biplot shows PC2 and PC3 components of each individual from control or ACeD patient groups; arrows indicate the loadings of the 5 bacteria most associated with PC2 and PC3.

(K) Box plots of absolute abundances of mucosal *Neisseria* in biopsies from control or ACeD patients. *, $P < .02$ Kruskal-Wallis.

(L) Numbers of control or ACeD patients with detectable or undetectable levels of the indicated bacteria.

(M) Heatmap shows the scaled effect size of each of the bacteria from (J) on GATA4 expression and ssGSEA scores of the indicated pathways in controls, compared to ACeD patients. · $P < 0.1$, * $P < 0.05$, t-test.

**** $P < 0.0001$, *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, · $P < 0.1$, ns $P > 0.05$, Fisher's exact test (C), Wilcoxon rank test (D, H), Kruskal-Wallis (I, K), t-test (M)