Supplemental information

GATA4 controls regionalization of tissue immunity and commensal-driven immunopathology

Figure S1. GATA4 controls regionalization of tissue metabolism and immunity

A. Relative expression of GATA4 in different tissue sections.

B. Principal component analysis (PCA) showing variation in Jejunum and Ileum between WT and GATA4ΔIEC.

C. Heatmap of selected metabolic genes and their expression in WT and GATA4ΔIEC in Jejunum and Ileum.

D. Heatmap showing gene expression patterns for WT and GATA4TG in Jejunum and Ileum.

E. Bar graph showing % IFNγ and % IL-17 in CD8a+ ileum between SPF and GF conditions.

F. Bar graph showing % IFNγ and % IL-17 in CD4+ ileum between SPF and GF conditions.

G. Enriched biological processes in SPF and GF conditions for WT and GATA4ΔIEC.

H. Venn diagrams showing the number of genes regulated by microbiota in a dependent or independent manner.
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 GATA4ΔIEC 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 GATA4ΔIEC 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 IFNγ+ cells among CD8αβ+ T cells (E), or of IL-17a+ cells among CD4+ T cells (F) in the ileum of SPF and GF WT and GATA4ΔIEC 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 IFNγ and IL-17 gene modules (right) from jejunum of SPF and GF WT and GATA4ΔIEC 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± 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, log₂ fold change) in GATA4ΔIEC versus WT mice of different bacteria taxa (dots), and their statistical significance (y axis, –log₁₀ 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 GATA4ΔIEC 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 GATA4ΔIEC 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 GATA4ΔIEC 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.
Figure S3. GATA4 controls the regionalization of tissue immune responses through commensal and pathogenic bacteria.

(A, B) Frequencies of IFNγ+ cells among CD8αβ+ T cells (A), and of IL-17a+ cells among CD4+ T cells (B) in the jejunum of WT and GATA4ΔIEC mice with different microbiota (x axis), i.e., SPF, GF, ASF, WT microbiota (no SFB), WT microbiota (with SFB), or GATA4ΔIEC microbiota (with SFB). N = 3-6 mice/group.
(C) Frequency of RORγt+ FOXP3- cells among transferred (CD45.1+ CD4+ Vβ14+ 7B8+ ) SFB specific T cells in the MLNs draining the jejunum and ileum of WT and GATA4ΔIEC 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 vβ14 TCR among transferred CD4+ CD45.1+ 7B8+ T cells in 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 IFNγ+ and IL-17a+ CD4+ LP T cells from the jejunum of GATA4ΔIEC 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 IFNγ+ CD4+ T cells which are summarized (right). Mice were analyzed 5 days after infection. N= 4-5 mice/group.

(H, I) Frequencies of IFNγ+ CD8αβ+ IEL (H), or IL17a+ CD4+ LP (I), from the jejunum of GATA4ΔIEC 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±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 GATA4ΔIEC mice 10 days after infection. N = 4-5 mice/group. *** P<0.001, t-test.
(B) Percent survival of WT and GATA4ΔIEC isotype treated, αIFNγ, or αIL-17a treated mice 0–20 days post *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 GATA4ΔIEC isotype treated, or αTNFα 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± 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 GATA4ΔIEC mice. N= 6 mice/group.

(G) Concentration of IgA, as measured by ELISA, in jejunal content of GF WT and GATA4ΔIEC 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 GATA4ΔIEC 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 GATA4ΔIE jejunum (K), or WT ileum versus WT jejunum and GATA4TG 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, GATA4ΔIEC vehicle-treated, and GATA4ΔIEC RA-treated mice. *P* = 0.69, t- test, comparing GATA4ΔIEC vehicle-treated and GATA4ΔIEC 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 jejunal-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)