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RESEARCH ARTICLE

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Zip14 (Slc39a14) metal transporter dysfunction leads to *Akkermansia* dysbiosis and inflammation in intestinal epithelium via epigenetic changes

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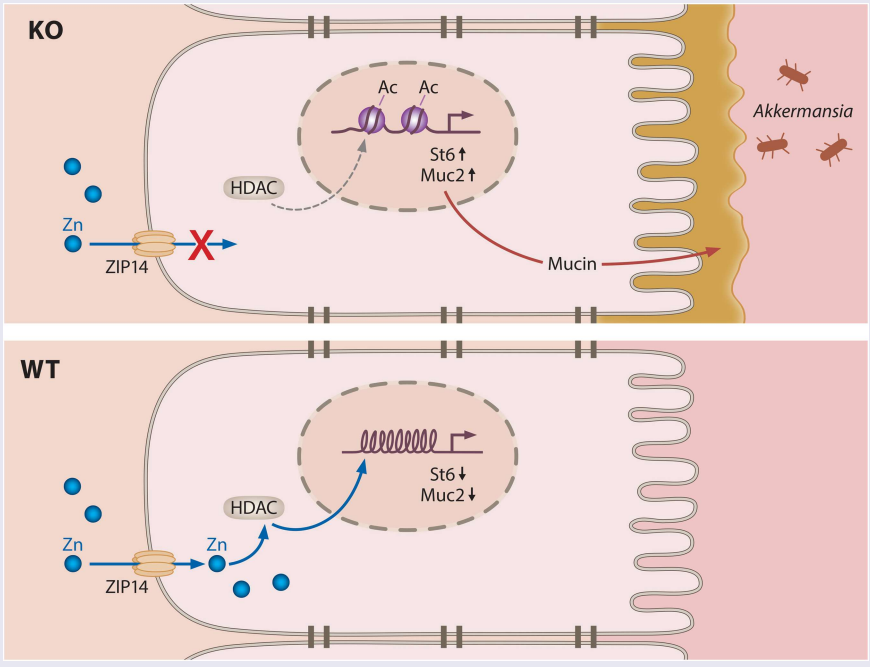
ABSTRACT

Zinc is recognized as an important component of immune function in animals, operating through many different mechanisms. Metal transporter Zip14 (Slc39a14) is up-regulated during pro-inflammatory conditions and increases zinc uptake into cells, including enterocytes. As reported here, global *Zip14* deletion in mice leads to excessive intestinal mucus accumulation and markedly increased fecal levels of the enteric microbe *Akkermansia muciniphila*. RNA sequencing showed up-regulation of a relatively small number of genes expressed in enterocytes isolated from *Zip14* knockout (KO) mice. Compared to wild-type (WT) mice. Among the most up-regulated genes were *St6galnac1* (ST6) and *Muc2*, both of which are involved in the synthesis of intestinal mucin. ATAC sequencing and ChIP assays using NF-κB antibodies demonstrated that open chromatin is the likely cause of the increased expression of these mucin-forming proteins. The differences in fecal *Akkermansia* abundance, inflammatory cytokine expression, and *St6* and *Muc2* expression were alleviated in KO mice when either orally dosed with pasteurized *Akkermansia* or provided a single oral gavage of zinc. Collectively, these experiments show that zinc delivery via Zip14 is necessary to limit chromatin accessibility for specific regulatory factors that influence mucin production and cause dysbiosis. These findings represent an example of a genome–nutrient–microbial interrelationship.



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1. Introduction

A variety of dietary components have been shown to influence commensal microbial populations of the gastrointestinal tract. The composition of this resident microbiota can profoundly impact nutrient utilization and metabolic processes.[1-3] Most attention has focused on macronutrients, with studies documenting both beneficial and adverse effects.[4-7] Much of this work has centered on the colonic microbiota, with comparatively less attention given to the small intestine.

Influences of micronutrients, particularly trace minerals, on intestinal microbiota have received limited attention.[8,9] Nevertheless, interactions between individual microorganisms and specific metals such as iron, manganese, and zinc are well recognized. Many of these interactions involve pathogenic organisms.[10,11] Ingested zinc has been shown to alter microbial populations.[12,13]

Our research has focused on the metabolic and functional roles of the zinc transporter Zip14, using mice with global (Zip14 KO) and intestinal epithelial cell-specific Zip14 (Zip14Δ^{IEC}) ablations.[14,15] Phenotypes associated with these deletions include systemic endotoxemia, elevated expression of multiple acute phase genes, decreased expression of specific major histocompatibility complex II (MHCII) genes, intestinal inflammation, and impaired barrier function. Several of these defects are reversible with modest zinc supplementation.

Notably, we have consistently observed overgrowth of *Akkermansia muciniphila* in fecal contents of mice with *Zip14* ablation.[16,17] Concurrent changes were not observed in other enteric microbial species (15). Shannon plots of α diversity supported the overabundance of *Akkermansia*. Importantly, this dysbiosis occurred under steady-state conditions in mice fed a natural-component chow diet, without dietary manipulation.

To identify mechanisms underlying these observations, we performed RNA-seq analysis to determine genes differentially expressed following *Zip14* deletion.[16] Relatively few genes within the intestinal epithelial cell (IEC) transcriptome were altered. Among down-regulated genes were major histocompatibility complex II (MHCII) genes.[16] Histone deacetylase (HDAC) activity was concurrently reduced following *Zip14* loss. Because most HDAC enzymes are zinc-dependent, we hypothesized that decreased HDAC activity reflects a localized intracellular zinc deficiency. Supporting this hypothesis, zinc uptake is reduced in IECs lacking *Zip14*.[15,16]

Zinc-dependent HDAC enzymes contribute to epigenetic chromatin regulation.[18] Using ChIP assays and ATAC sequencing, we demonstrated chromatin remodeling at promoter sites of MHCII genes, providing mechanistic insight into altered gene expression patterns observed in IECs from *Zip14* knockout mice.[15,16]

In the present study, we report experiments identifying IEC genes that are up-regulated following targeted deletion of *Zip14*. Our findings indicate that *Zip14* loss induces epigenetic events that regulate the expression of genes involved in intestinal mucus production. These changes would be expected to favor expansion of mucus-utilizing *Akkermansia* in the gastrointestinal tract. Collectively, these experiments demonstrate that host genetics can modify nutrient-microbial interactions through epigenetic mechanisms, resulting in dysbiosis.

2. Materials and methods

2.1. Animal husbandry and treatments

Zip14 knockout mouse strains used in these studies have been described previously.[16,18] All backcrosses were to C57BL/6 mice with generations > F20. Mice were used as young adults (8–16 weeks). Maintenance consisted of shoebox caging, a 12/12 light/dark cycle, and *ad libitum* access to natural component diet (Teklad 2918) and tap water.

For fecal collections of 1 or 2 d, mice were placed individually in new cages with fresh bedding.

Mice were euthanized by cardiac exsanguination under isoflurane anesthesia. Tissues were removed and serum was prepared.

In one study, mice maintained as above received zinc (zinc sulfate; 500 μmol/kg body weight) as a single oral gavage (200 μL), 44 h before tissues and feces were obtained.[19] Control mice received saline.

In another series, mice received pasteurized *Akkermansia muciniphila* (Vitamatic; Raritan, NJ) as a suspension containing 10^9 active fluorescent units (AFU) in 200 μL PBS by oral gavage daily for two weeks (20). Pasteurized *A. muciniphila* is known to have physiological effects.[20-22] Control mice received PBS daily for two weeks.

Mice used to produce organoids were fed the natural component diet.

Protocols were approved by the University of Florida Institutional Animal Care and Use Committee (No. 202007015).

2.2. Isolation of intestinal epithelial cells

Cells used for RNA-seq were purified as described previously.[16] For all other data, IECs were obtained from the first 20 cm of proximal small intestine, excised and perfused with ice-cold buffer (Buffer B: 10 mM EDTA, 10 mM HEPES, 0.9% NaCl, phosphatase/protease inhibitor mix).

Intestinal sections were cut open, placed in Buffer B for 15 min, then transferred to PBS and vortexed for 30 sec each, before moving the intestinal sections to a new tube of PBS. This was done 3 times. Cells from the three suspensions were combined for each mouse and centrifuged (1,000 × *g*, 10 min, 4 °C). The pellet was re-suspended in fresh PBS and centrifuged again to wash. IEC pellets were used immediately or frozen at −80 °C.

2.3. Preparation and culture of intestinal organoids

Twenty centimeters of proximal small intestine from three mice of each genotype were excised, perfused with PBS, cut into 5 mm strips, and placed in cell dissociation reagent (StemCell Technologies, Inc.). Cell domes were embedded in Matrigel (Corning) and plated in 6-well plates as described previously.[15]

For rescue experiments, cultures were incubated for 10 d with ±15 μM Zn (as $ZnSO_4$) added as fresh medium for the final 7 d.

2.4. Quantitative PCR

Total RNA was extracted using TRI Reagent (MRC) with homogenization via Bullet Blender (Next Advance) using RNase-free zirconium oxide beads. RNA quality and concentration were assessed spectrophotometrically (NanoDrop, Thermo Fisher).

First-strand cDNA was synthesized using High-Capacity RNA-to-DNA reagents (Applied Biosystems). Real-time PCR was performed using a QuantStudio 3 system (Thermo Fisher). Detection utilized EXPRESS SYBR GreenER Supermix with ROX or TaqMan Gene Expression Assays. Primer/probe sequences have been published[15,23] or are listed in SI Table 1.

Reactions were run in duplicate. Data were derived from at least two independent RNA preparations. 18S rRNA served as the normalizer and relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method.

2.5. Western analyzes

IECs were homogenized in RIPA buffer (150 mM NaCl, 50 mM Tris-HCl pH 7.4, 1 mM EDTA, 0.1% Triton X-100, 0.1% Na deoxycholic acid, 0.1% SDS) with protease and phosphatase inhibitors. Protein concentration was measured using the Pierce BCA Protein Assay (ThermoFisher).

Proteins were separated using 10% mini-PROTEAN TGX gels (BioRad) and transferred to 0.45 μm nitrocellulose membranes (Amersham) for 1 h at 60 V. Ponceau Red staining verified transfer uniformity. Using the PageRuler Plus Prestained Protein Ladder (ThermoFisher), membranes were sectioned as needed and probed with antibodies. Detection was by chemiluminescence (Protein Simple). Original images are available upon request. Antibody sources are listed in SI Table 1.

2.6. Chromatin immunoprecipitation assays

IECs were cross-linked in PBS containing 1% formaldehyde for 15 min at room temperature. Crosslinking was quenched with glycine buffer. Cells were lysed and chromatin were fragmented by sonication (Bioruptor, Diagenode) to 200–700 bp fragments.

Immunoprecipitations were performed with ChIP-grade antibodies (SI Table 1). qPCR used SimpleChip Universal qPCR Master Mix (Cell Signaling) with primers listed in SI Table 1.

2.7. RNA- and ATAC-sequencing

RNA from IECs was sequenced on an Illumina NovaSeq (2 × 100 bp). ATAC-Seq was performed by MedGenome (Foster City, CA) using an Illumina HiSeq 2000. Methods were reported previously (16). Data are archived at GEO (GSE280523).

2.8. Fecal collection and analysis

Fecal DNA was extracted using the Quick-DNA Fecal/Soil Microbe 96 Kit (Zymo Research). DNA was quantified spectrophotometrically. Relative abundance of *Akkermansia* was determined by qPCR using species-specific primers (SI Table 1).

Additionally, 16S rRNA sequencing was performed commercially (CosmosID; Germantown, MD).

2.9. Statistical analysis

Data are presented as means ± SE of biological replicates. Experiments were replicated. GraphPad Prism 10.0 was used for statistical analyzes. Student's *t* test and ANOVA were used for most analyzes. The Wilcoxon rank order test was used for fecal parameters and oral *Akkermansia* supplementation. Statistical significance was defined as $P < 0.05$.

3. Results

3.1. Zip14 deletion leads to dysbiosis and intestinal mucus accumulation

Global *Zip14* deletion elevated transcripts for *Il-6* in IECs and increased circulating IL-6, characteristics of local and systemic low-grade inflammation, respectively (Figure 1A). Sequencing of 16S rRNA revealed that microorganisms of the species *Akkermansia* were markedly more abundant in fecal microbial populations from *Zip14* KO mice compared to WT controls (SI Figure 1). Moreover, *Zip14* KO mice exhibited overabundance of *Akkermansia muciniphila* in feces when measured by qPCR using two independent primer/probe sets (Figure 1B).

PAS/Alcian Blue staining of perfused ileum cross-sections demonstrated equivalent goblet cell abundance in WT and KO mice (Figure 1C). However, despite perfusion prior to fixation, increased Alcian blue staining material, presumed to be mucin, was apparent in KO intestines.

3.2. RNA-seq shows Zip14 ablation produces up-regulation of mucus-producing genes

RNA-seq was used to profile highly purified IECs from *Zip14* KO mice to investigate loss-of-function effects[15] [NCBI GEO: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE280523]. The heat map shows the transcript levels of individual WT and KO mice in specific up-regulated genes identified by RNA-seq (Figure 2A). These levels confirm the up-regulation of these genes in mice with the *Zip14* ablation. Among the 226 genes significantly up-regulated in KO IECs, *St6galnac1* (*St6*) was the most over-expressed IEC-related gene, with a fold change of +9.2 (Figure 2B). As ST6 is a key enzyme in mucin biosynthesis,[24] this up-regulation may explain mucus accumulation in KO mice.

The MA plot demonstrated additional up-regulated genes including *Muc2*, *Mcoln3*, *H2-bl*, and *Cfi* (Figure 2B). qPCR validation using independent RNA preparations confirmed significant elevation of *St6* in KO IECs (Figure 2C). This differential expression was not observed in RNA from whole intestine, confirming IEC purity.

Similarly, qPCR confirmed up-regulation of *Muc2* and *Cldn2*, while *Cldn1* was down-regulated (Figure 2C). These genes are associated with mucus production, intestinal permeability, and epithelial

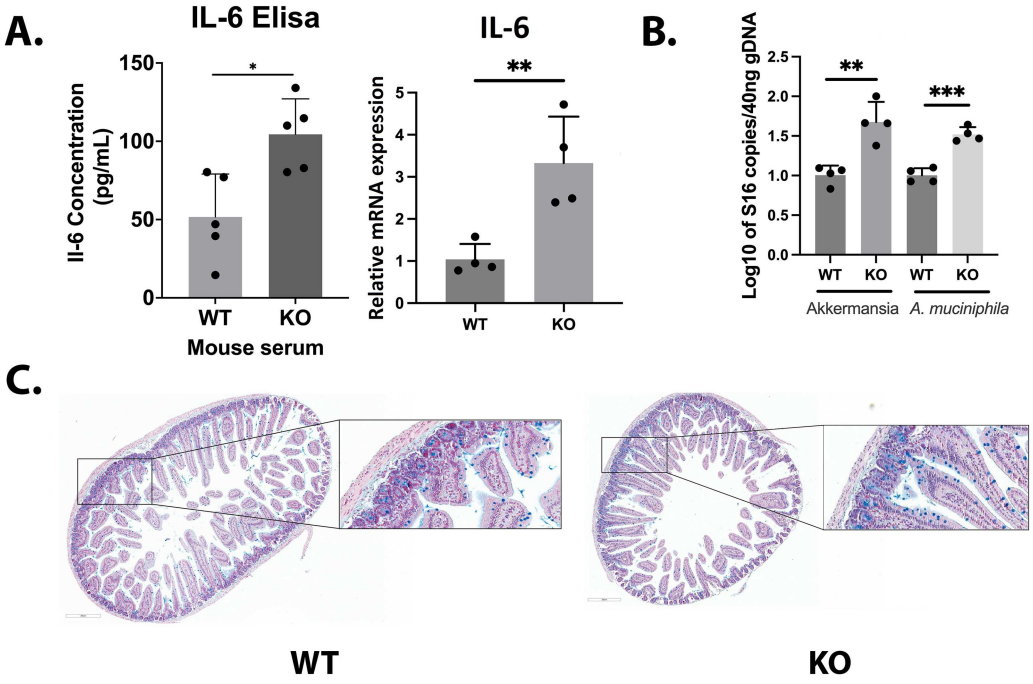

Figure 1. IL-6, intestinal mucus, and *Akkermansia* fecal excretion changes produced by *Zip14* ablation. A: Serum IL-6 levels are increased and *Il-6* mRNA expression is up-regulated in intestinal epithelial cells (IECs) from *Zip14* KO mice. Values are means ± SE, $n = 4 - 5$ /group, $^{*}P < 0.05$, $^{**}P < 0.01$. B: Differences in microbial species in feces from *Zip14* WT vs. KO mice detected by 16S DNA sequencing. Values are means ± SE, $n = 4$ /group, $^{**}P < 0.01$, $^{***}P < 0.001$. C: Alcian blue staining of cross-sections of ileum from WT and KO mice showing Goblet cells and mucin.

defense. *Fabp6* served as a sentinel transcript.[15,16] Given that *Fabp6*-producing goblet cells represent a minor IEC fraction,[25] transcripts for *Fabp6*, *Muc2*, and *St6* were likely derived primarily from enterocytes.

Western blot analysis demonstrated modest but variable increases in ST6GALNAC1 and MUC2 proteins in KO IEC lysates (Figure 2D and E). Collectively, up-regulation of ST6 and MUC2 likely explains the increased intestinal mucin observed with *Zip14* ablation.

3.3. *Akkermansia* and zinc supplementation reverse the effects of zip14 ablation

Given the overabundance of *Akkermansia* in feces of *Zip14* KO mice we evaluated expression of specific genes following daily oral gavage with pasteurized *Akkermansia* (10^9 AFU) as indicated by +AKK in the figures. *Il-6* remained elevated in KO mice and was not normalized by supplementation (Figure 3A).

However, *St6* and *Muc2* expression levels in KO IECs were restored to WT baseline levels following supplementation (Figure 3B), suggesting beneficial transcriptomic modulation. Tight junction transcripts *Cldn1*, *Zo1*, *Jam2*, and *Zo3* were similarly normalized, although *Cldn2* remained elevated (Figure 3C).

MHCII genes *H2-Ab1* and *H2-Aa* were depressed in KO mice but maintained at WT levels with supplementation (Figure 3D).

Oral zinc gavage was used to acutely increase zinc availability. As previously shown, this model induces transient serum zinc elevation and metallothionein expression.[19] Unexpectedly, zinc drastically up-regulated *St6* expression (Figure 4A), despite the absence of apparent metal response elements in the promoter. In contrast, elevated *Muc2*, *Cldn2,* and *Il6* expression, and reduced *Cldn1* expression in KO IECs normalize following zinc gavage (Figure 4A). Zinc supplementation did not alter the elevated fecal *Akkermansia* DNA levels in the KO mice when either PCR probes were used (Figure 4B).

Organoids derived from WT and KO IECs recapitulated increased *St6*, *Muc2*, and *Il-6* transcripts in KO cultures (Figure 4C). Zinc treatment prevented these increases in KO organoids, suggesting that lack of ST6 normalization in intact mice may involve microbiota-dependent factors.

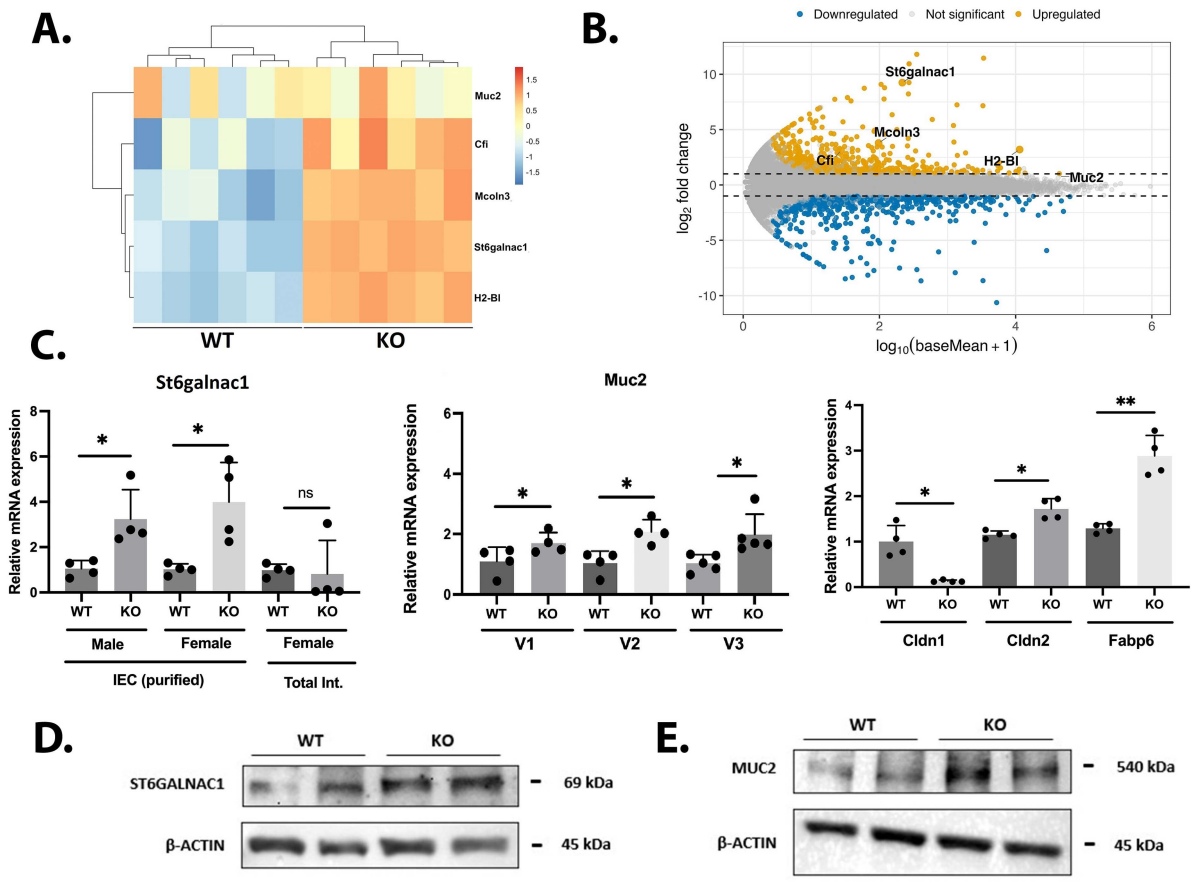

Figure 2. Differential up-regulation of specific genes in intestinal epithelial cells (IECs) of mice with *Zip14* ablation. A: Heat map showing expression of highly up-regulated genes as detected using RNA-sequencing. Each column represents data from one mouse of WT or KO genotypes. B: MA plot of RNA-seq analysis of IECs from WT and KO mice. C: Confirmation of differential expression of specific genes identified by RNA-seq using qPCR. *St6* expression in IECs and total intestine of WT and KO mice. Three individual primer sets were used for *Muc2* measurements and are designated v1, v2, and v3. *Fabp6* was used as a control for an up-regulated gene. Values are means ± SE, $n = 4$ /group, $^{*}P < 0.05$, $^{**}P < 0.01$. D and E: Western analysis showing differential expression of ST6 and MUC2 protein in WT vs. KO mice.

3.4. ChIP assay and ATAC-seq demonstrate increased NF-κB occupancy at the St6 promoter

Given the inflammatory phenotype, ChIP assays were performed using NF-κB antibodies. NF-κB occupancy at multiple sites within the *Muc2* and *St6* promoters was markedly increased in KO chromatin compared to WT (Figure 5A).

ChIP analysis of chromatin revealed enrichment of active marker H3K4 and alterations in repressive marker H3K27 consistent with enhanced chromatin accessibility (Figure 5A).

ATAC-Seq analysis further supported chromatin remodeling. Comparing RNA-seq to ATAC accessibility ratios for *St6* and *Eno1b* demonstrated that chromatin openness correlated with divergent expression patterns in KO IECs (Figure 5B). Increased promoter peak intensity at three distinct *St6* regions was observed in KO IECs relative to WT (Figure 5C). A similar but less pronounced accessibility pattern was noted for *Muc2*.

Collectively, these data demonstrate that Zip14 deletion enhances chromatin accessibility and NF-κB occupancy at mucin-related gene promoters, particularly *St6*, providing a mechanistic explanation for increased mucus production.

4. Discussion

Diet constituents have been demonstrated to influence the composition of the resident microbial population of the gastrointestinal tract of experimental animals and humans.[1-3] Referred to as nutrient-microbial

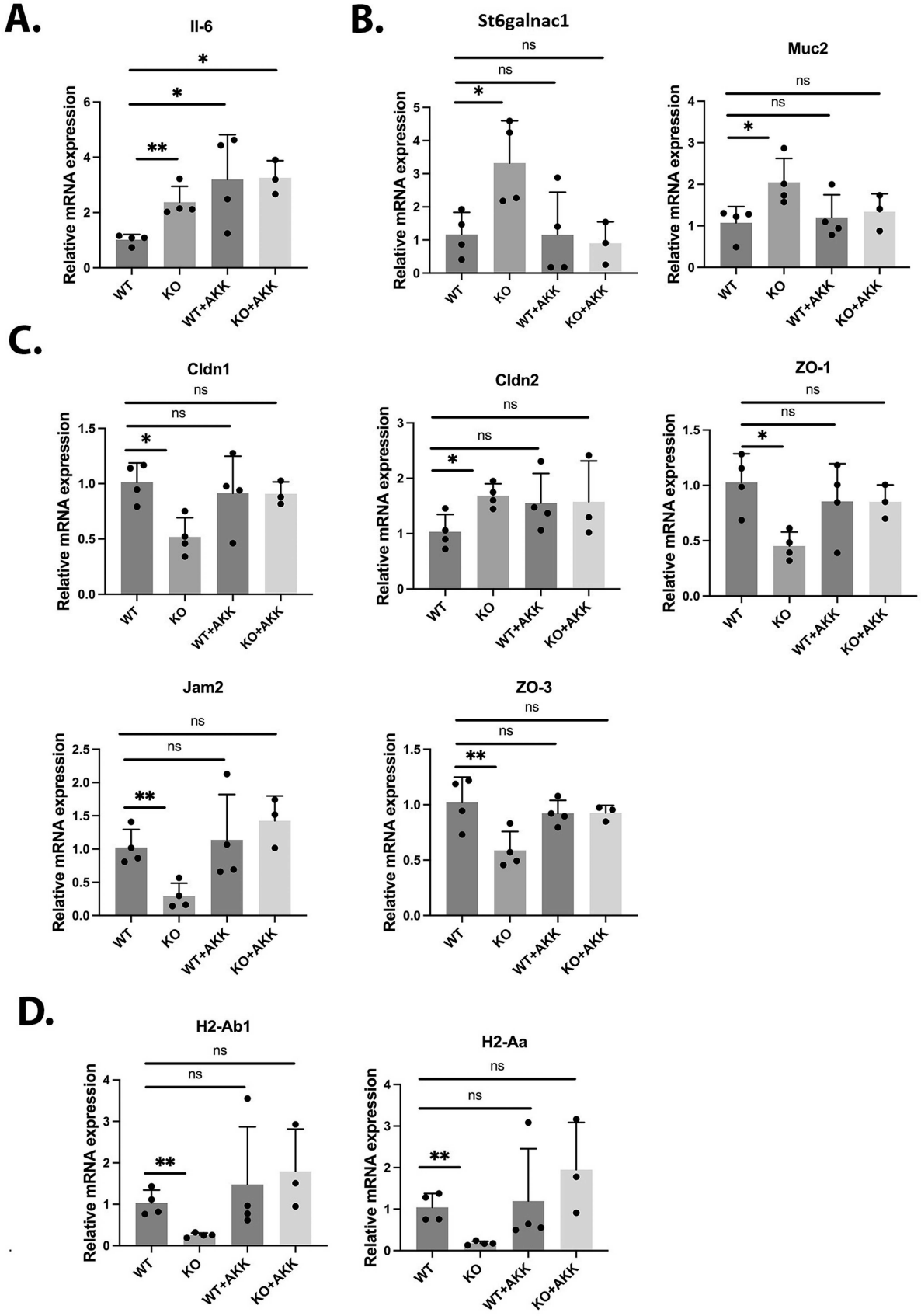

Figure 3. Oral *Akkermansia* supplementation given by daily gavage for two weeks to mice reverses many transcript differences in intestinal epithelial cells produced by *Zip14* deletion. A: *Il-6*. B: *St6* and *Muc2*. C: *Cldn1*, *Cldn2*, *Zo1*, *Jam2*, and *Zo3*. D: *H2-Ab1* and *H2-Aa*. Values are means ± SE, $n = 4$ /group, $^{*}P < 0.05$, $^{**}P < 0.01$.

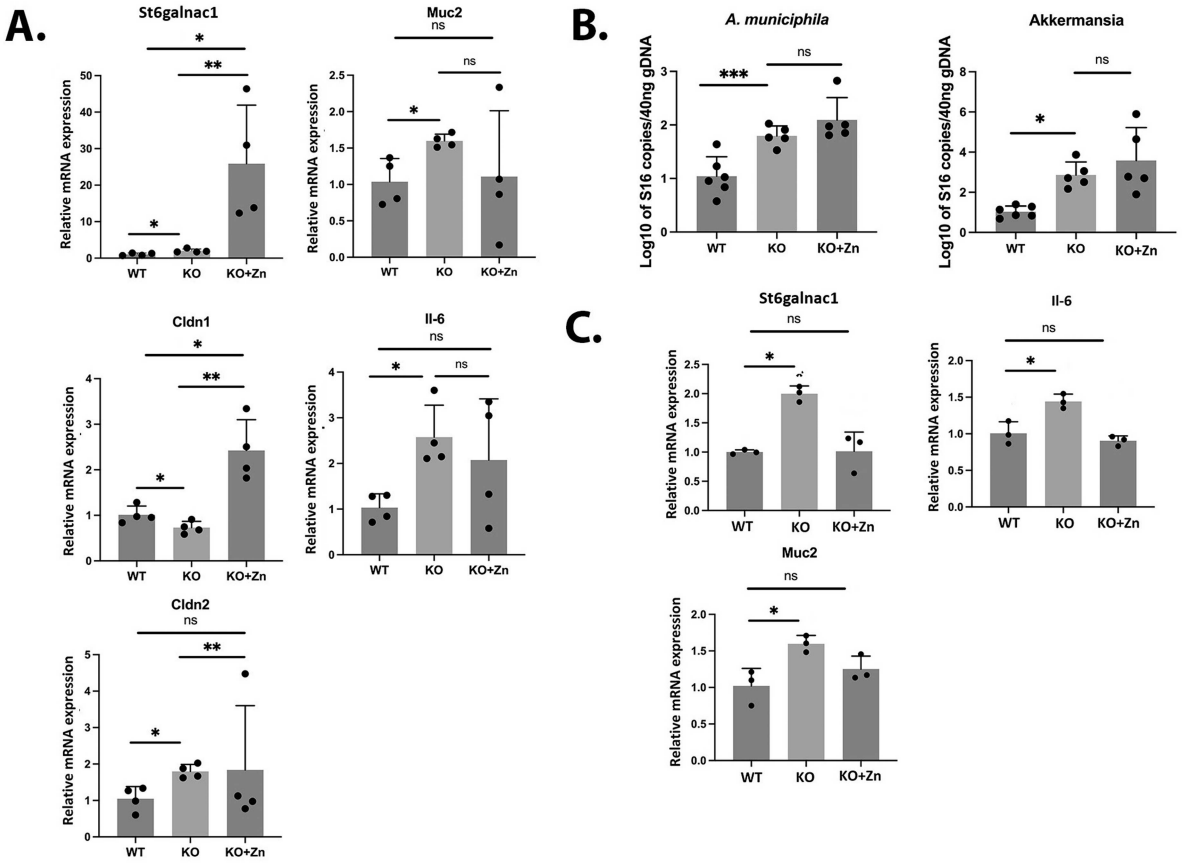

Figure 4. Zinc supplementation given as an acute dose reverses many transcripts in intestinal epithelial cells produced by *Zip14* deletion. A: IECs isolated from mice given zinc by oral gavage: *St6*, *Muc2*, *Cldn1*, *Il-6*, and *Cldn2*. Values are means ± SE, $n = 4$ /group, $^{*}P < 0.05$, $^{**}P < 0.01$. B: Differences in microbial species in feces detected by 16S DNA. Values are means ± SE, $n = 5 - 6$ /group, $^{*}P < 0.05$, $^{***}P < 0.001$. C: Intestinal organoids derived from WT and KO mice and cultured for 7 d with ± 15 μM zinc: *St6*, *Il-6*, *Muc2*. Values are means ± SE, $n = 3$ organoids/group, $^{*}P < 0.05$.

interactions, these interactions may have profound effects on host physiology, including metabolic, neurologic, and inflammatory disorders.[26-28] The experiments reported here represent an example of a nutrient-genome-microbial interaction. Specifically, genetic deletion of the *Zip14* gene, responsible for zinc transport in intestinal epithelial cells, leads to spontaneous over-abundance of the intestinal microbe *Akkermansia*, without dietary manipulation.

We have demonstrated this interaction in mice using both global and enterocyte-specific *Zip14* ablation models.[15,16] Importantly, *Zip14* ablation presents as a phenotype characterized by increased intestinal permeability, intestinal inflammation, and endotoxemia. The immunological profile associated with *Zip14* deletion in IECs, including elevated IL-6, may result from direct effects on inflammatory gene regulation and/or from microbial influences on the intestinal epithelium.

The zinc transporter ZIP14 is localized at the basolateral surface of intestinal epithelial cells in mice.[14] Because its expression decreases from the proximal to distal gastrointestinal tract, our studies have focused on the proximal small intestine and the contribution of ZIP14 to intestinal homeostasis.[15,16] Acute zinc supplementation, both *in vivo* and *in vitro*, reverses several metabolic signatures of *Zip14* ablation. We propose that this response reflects correction of a localized intracellular zinc deficiency.

Supporting this hypothesis, previous studies in IECs demonstrated that *Zip14* ablation reduces labile intracellular zinc and histone deacetylase (HDAC) activity.[15,16] HDACs comprise a family of predominantly zinc-dependent enzymes that regulate chromatin acetylation and transcription factor (TF) accessibility to gene promoters. Because HDAC activity depends on zinc availability,[17] defective zinc transport would be expected to alter HDAC kinetics, chromatin openness, and gene expression. Indeed, *Zip14*

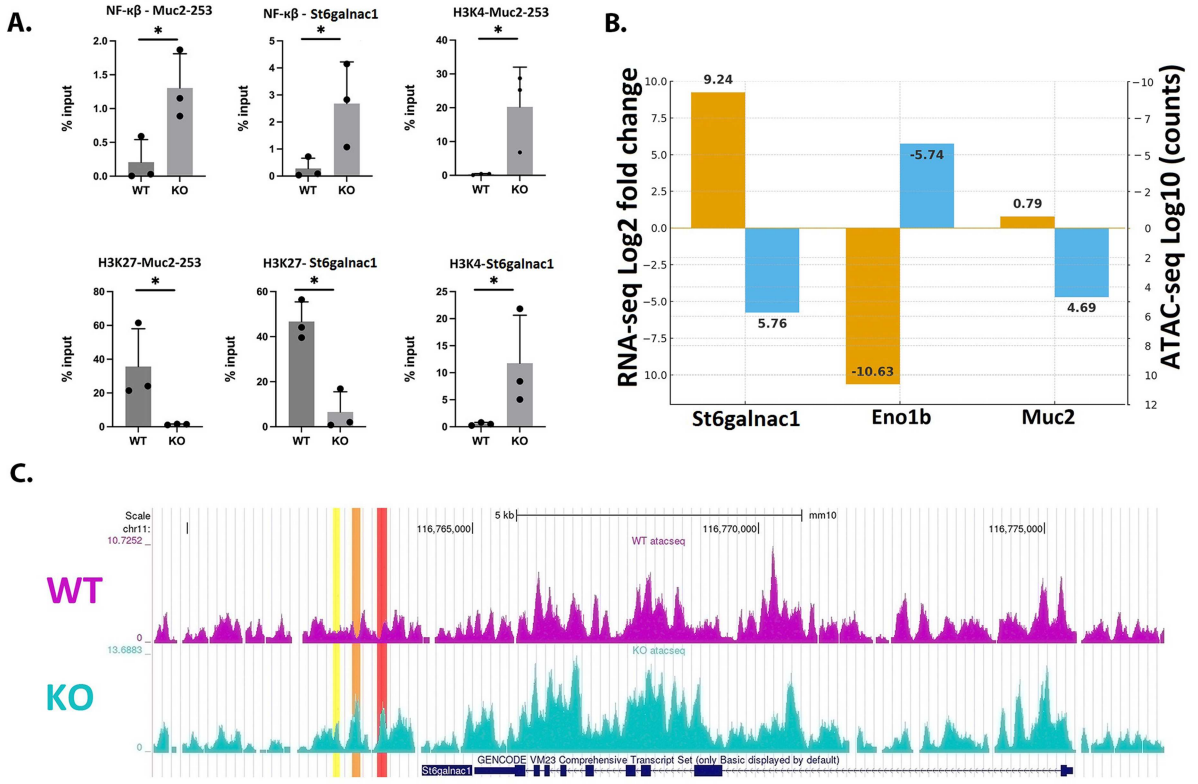

Figure 5. Differential regulation of *St6* and *Muc2* in intestinal epithelial cells with *Zip14* ablation is related to increased transcription factor occupancy. A: ChIP assays for *St6* and *Muc2* promoters using antibodies NF-κB, H3K4, and H3K27. Values are means ± SE, $n = 3$ /group, $^*P < 0.05$. B: Bar graph showing the relationship between *St6*, *Eno1* and *Muc2* obtained by RNA-seq vs. chromatin accessibility assessed by ATAC-seq. *St6* and *Eno1* are among the most up-regulated and down-regulated genes, respectively. Their differential expression represents the result of more open and closed chromatin, respectively, caused by *Zip14* deletion. Similarly, the differential expression for *Muc2* can be related to chromatin accessibility. **C:** Differential peak densities in the regulatory region of *St6* from chromatin of WT and KO mice. The open chromatin regions used were: 116,763,328–116,763,504 (red), 116,762,846–116,763,045 (orange), and 116,762,557–116,762,621 (yellow).

ablation produces dysregulated expression of a limited but biologically significant set of genes associated with IEC homeostasis.

Mechanistically, the link between *Zip14* deletion and increased *Akkermansia* abundance can be explained at the molecular level. The striking upregulation of *St6galnac1* suggests enhanced mucin biosynthesis. Concomitant upregulation of *Muc2* and increased mucin accumulation, as demonstrated by Alcian blue staining, support greater mucin abundance in KO mice. Notably, *Akkermansia* utilizes mucin as an energy source,[24] providing a plausible explanation for its overgrowth.

Inflammatory signaling through NF-κB has been linked to ST6 activity.[29] Our ChIP data demonstrate increased NF-κB binding at the *St6* and *Muc2* promoters in IEC chromatin from KO mice. ATAC-Seq analysis further shows increased chromatin accessibility in relevant promoter regions. Together with previous findings,[15,16] these data support an epigenetic mechanism underlying the phenotype. Specifically, reduced HDAC activity due to intracellular zinc deficiency increases promoter acetylation and transcription factor accessibility. Alternatively, altered acetylation of NF-κB subunits themselves may influence promoter binding. These findings align with emerging evidence that specific zinc transporters mediate phenotypic effects through zinc-dependent epigenetic mechanisms regulating gene expression.[17,30] We also cannot exclude roles for newly described zinc-dependent regulators such as Zincore.[31] Although microbial metabolites such as short-chain fatty acids[32] could contribute to *Akkermansia* overgrowth, this explanation is less likely since zinc supplementation reverses key phenotypes in organoid cultures lacking microbiota.

There is substantial evidence that *Akkermansia* supplementation confers beneficial effects in metabolic and intestinal disorders,[27,33,34] although intestinal damage associated with excessive exposure has also been reported.[35,36] In our studies, supplementation normalized elevated *St6* and *Muc2* expression and restored tight junction transcripts (*Cldn1*, *Zo1*, *Jam2*, *ZO3*) to WT levels. Expression of MHCII genes *H2-Ab1* and *H2-Aa* were also normalized, potentially reflecting links between mucus regulation and food allergy.[26] These findings suggest a context-dependent beneficial effect of *Akkermansia*.

Dietary zinc has been shown to influence intestinal microbiota composition, including *Akkermansia muciniphila*.[37-39] Such effects are variable and influenced by diet composition and exposure duration. The spontaneous increase in *Akkermansia* observed with *Zip14* ablation suggests a host-driven response secondary to intracellular zinc deficiency. Further studies in human subjects are warranted, particularly given the tight homeostatic regulation of dietary zinc c.[40]

In summary, our findings demonstrate that host genetics can profoundly influence intestinal microbiota through the mechanistic restriction of transport of an essential dietary micronutrient. Deletion of *Zip14* reduces intracellular zinc availability, lowers HDAC activity, alters chromatin accessibility, and results in differential gene expression in intestinal epithelial cells. Downstream consequences include over-expression of mucin-related genes, creating an environment favorable for mucin-utilizing microorganisms such as *Akkermansia*. These alterations may compromise barrier integrity and intestinal health.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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None.

Author contributions

CRediT: **Felix R. Jimenez-Rondan:** Conceptualization, Data curation, Formal analysis, Methodology, Validation, Visualization, Writing – review & editing; **Courtney H. Ruggiero:** Data curation, Formal analysis, Writing – review & editing; **Robert J. Cousins:** Investigation, Methodology, Writing – original draft, Writing – review & editing.

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Data availability statement

The RNA-seq and ATAC-seq datasets generated in this study are publicly available at the NCBI Gene Expression Omnibus (GEO) under accession number GSE280523 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE280523).

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