miR-802 regulates Paneth cell function and enterocyte differentiation in the mouse small intestine

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The intestinal epithelium is a complex structure that integrates digestive, immunological, neuroendocrine, and regenerative functions. Epithelial homeostasis is maintained by a coordinated cross-talk of different epithelial cell types. Loss of integrity of the intestinal epithelium plays a key role in inflammatory diseases and gastrointestinal infection. Here we show that the intestine-enriched miR-802 is a central regulator of intestinal epithelial cell proliferation, Paneth cell function, and enterocyte differentiation. Genetic ablation of mir-802 in the small intestine of mice leads to decreased glucose uptake, impaired enterocyte differentiation, increased Paneth cell function and intestinal epithelial proliferation. These effects are mediated in part through derepression of the miR-802 target Tmed9, a modulator of Wnt and lysozyme/defensin secretion in Paneth cells, and the downstream Wnt signaling components Fzd5 and Tcf4. Mutant Tmed9 mice harboring mutations in miR-802 binding sites partially recapitulate the augmented Paneth cell function of mice lacking miR-802. Our study demonstrates a broad miR-802 network that is important for the integration of signaling pathways of different cell types controlling epithelial homeostasis in the small intestine.
MicroRNAs (miRs) are essential post-transcriptional repressors of mRNA targets influencing complex gene networks that regulate fundamental processes of cell differentiation, proliferation, development, and homeostasis. Genetic studies in mice have been crucial in linking miRNA function to developmental, cellular, physiological, and behavioral phenotypes. Importantly, many miRNA knock-out mouse models exhibit differential responses to disease or injury and some have revealed altered susceptibility to infection.

miRs are grouped into families based on their targeting properties, which depend primarily on the base pair identity of their extended seed region (i.e., miRNA nucleotides 2–8). Of the ~500 canonical miRNA genes that have been identified in the human genome, miR-802 is a unique member of 62 seed families that are conserved from bony fish to vertebrates. Despite its evolutionary conservation, our biological understanding of miR-802 function on a cellular and organismic level is scarce. MicroRNA-802 was first studied in the liver, where it was reported to be upregulated in insulin-resistant and obese states. Other studies have suggested that miR-802 may play a role in insulin secretion and have anti-oncogenic properties in organs of the GI tract. Furthermore, it has been shown that hepatic miR-802 levels are higher in females than males and that plasma miR-802 levels are increased in rats when treated with nephro- or hepatotoxins.

Intestines evolve in evolution as early as teleost fish, where they have an important role in increasing both the surface area and the effective length of the intestine for absorption of macro and micronutrients from food. The epithelium of the small intestine is organized into large numbers of self-renewing crypt-villus units. The base of each villus is surrounded by multiple epithelial invaginations, that contain highly proliferating stem cells, which sustain the self-renewal of the epithelium. Six differentiated epithelial cell types are distinguished in the intestine: the absorptive enterocytes, goblet cells, and enteroendocrine cells that secrete mucus and a variety of hormones, respectively, Tuft cells that may sense luminal contents, microfold (M) cells that oversee the Peyer’s patches, and Paneth cells that occupy the bottom positions in the crypt adjacent to intestinal stem cells. Paneth cells secrete bactericidal products such as lysozyme and defensins, play key roles in mucosal immunity, provide niche factors to support crypt base columnar cells (CBC), including epidermal growth factor (EGF), Wnt (WNT3A) and Notch ligands, and bone morphogenetic protein (BMP) inhibitor Noggin.

In this study, we describe an important role of miR-802 in the maintenance of intestinal homeostasis. We demonstrate that genetic deletion of miR-802 expression or mutations in miR-802 binding sites of a single target gene results in distinct phenotypes and leads to dysregulated gene networks influencing epithelial glucose uptake, proliferation, differentiation, and innate immunity.

**Results**

**miR-802 deletion in mice impairs intestinal glucose transport.**

Expression analysis of miR-802 levels in multiple tissues revealed that the jejunum had significantly higher levels than previously studied organs (Fig. 1a). A more detailed analysis of miR-802 in the entire gut revealed the highest levels in the upper small intestine (duodenum and jejunum), intermediate levels in the ileum and undetectable expression in the colon (Fig. 1b). Expression of miR-802 in the upper intestine was slightly higher in females than males, a finding that was previously reported in the liver. Since miRNA knockout phenotypes are most often observed in organs with high miRNA expression levels, we investigated mainly female mice with a global (referred to as mir-802KO) or intestine-specific deletion of mir-802 (Vil-Cre mir-802fl/fl) (Supplementary Fig. 1b, c). Given the prominent role of the jejunum in nutrient uptake and previous studies showing the involvement of mir-802 in glucose homeostasis, we first studied the effect of mir-802 ablation on glucose metabolism. Mice had similar body weight, blood glucose levels and, intraperitoneal glucose and insulin tolerance tests (IPGTT and IPITT, respectively) (Supplementary Fig. 1d–g). However, when mice were given an oral glucose load by gavage (oral glucose tolerance test, OGTT), we observed lower blood glucose levels in mir-802KO and Vil-Cre mir-802fl/fl mice, whereas IPGTT and IPITT were similar in mutant mice compared to littermates control animals (Fig. 1c–e, Supplementary Fig. 1g, h). These data were also confirmed in male mice. These results indicate that the ablation of mir-802 in the intestinal epithelium may be responsible for this effect by either influencing incretin secretion from enteroendocrine cells of the gut epithelium or by affecting glucose absorption. Measurements of plasma glucagon-like peptide 1 (GLP-1) and insulin after an oral glucose load revealed similar levels in Vil-Cre mir-802fl/fl and controls (Fig. 1f, g). Furthermore, expression of glucose-dependent insulino-tropic peptide (GIP) and GLP-1 as well as total GLP-1 content in jejunal tissue revealed no change (Fig. 1h, i). Having ruled out an incretin effect as the cause for the improved glucose tolerance, we next hypothesized that the glucose phenotype might be related to altered intestinal glucose absorption. We performed radioactive glucose uptake assays following oral administration of 14C-glucose in Vil-Cre mir-802fl/fl mice and mir-802fl/fl littersmates and measured postprandial 14C-glucose levels in the blood and tissues of the proximal and distal jejunum. 14C-glucose was markedly lower in the proximal jejunum of Vil-Cre mir-802fl/fl mice where most monosaccharides are absorbed; moreover, 15 min after gavage, 14C-glucose levels in the circulation were reduced in Vil-Cre mir-802fl/fl mice compared to controls (Fig. 1j–l). We also analyzed the expression of glucose transporters in isolated jejunal enterocytes and found a ~60% decrease of Glut2, Sglt1, and Glut5 transcripts in Vil-Cre mir-802fl/fl compared to mir-802fl/fl or Vil-Cre control mice (Fig. 1m, n). Western blotting of GLUT2 Vil-Cre mir-802fl/fl mice (Fig. 1o).

Together, these results demonstrate that mir-802 influences glucose tolerance by regulating intestinal glucose absorption through downregulation of intestinal glucose transporter expression.

**miR-802 regulates Paneth cell expansion and function.** To investigate if mir-802 ablation leads to abnormal phenotypes in the intestine beyond glucose transport, we first explored if morphological differences can be detected in mice lacking mirR-802. Crypt and villus sizes and the number of Goblet cells were similar in Vil-Cre mir-802fl/fl compared to mir-802fl/fl mice (Supplementary Fig. 2a, b). We next measured the relative miR-802 levels in different intestinal cell types such as Lgr5+ stem cells, Paneth cells, and enterocytes following FACS sorting. Levels of miR-802 were highest in Paneth cells (1.7–fold) compared to enterocytes, and much lower in Lgr5+ cells (Fig. 2a). Given the high mir-802 expression, we next analyzed Paneth cell numbers by immunofluorescence staining using the Paneth cell-specific marker lysozyme (LYZ). Interestingly, we found higher Paneth cell numbers per crypt in the jejunum of Vil-Cre mir-802fl/fl mice compared to control mir-802fl/fl animals, while no changes were detected in the proximal ileum (Fig. 2b, Supplementary Fig. 2c). Paneth cells in Vil-Cre mir-802fl/fl mice were located at the base of intestinal crypts with no indication of Paneth cell metaplasia (Fig. 2b). The increased Paneth cell numbers were also confirmed by FACS analysis of dispersed intestinal epithelial cells (Fig. 2c, Supplementary Fig. 2d), revealing a ~25% increase in Vil-Cre mir-802fl/fl compared to mir-802fl/fl mice. In contrast, enterocyte numbers were 25% lower in Vil-Cre mir-802fl/fl mice compared to littermates (Fig. 2d, Supplementary Fig. 2e).
were similar between groups (54% ± 7 vs. 55% ± 7, respectively). Successful FACS sorting of Paneth cells was validated by qRT-PCR measurements of Lyz and Defa (Supplementary Fig. 2e). Furthermore, expression analysis of isolated intestinal epithelial cells by RNA-seq and qRT-PCR revealed increased transcript levels of Lyz, angiogenin-4 (Ang4), phospholipase A2 (Pla2), and cryptdins (Defa 5, 20, 21, 22), which were all enriched in Paneth cells (Fig. 2d, e). Furthermore, the proteolytic processing enzyme MMP-7, which is required for the activation of defensins\textsuperscript{18}, was increased in mice with genetic mir-802 ablation (Fig. 2d). Also, secreted Reg3γ, which is also expressed in other gut epithelial cell types and helps to maintain the relative bacterial sterility on the epithelial surface of the jejunum\textsuperscript{19}, was also increased (Fig. 2d). This is consistent with a modest increase in NF-κB and Tnfa expression (Fig. 2f)\textsuperscript{20}. Together, these data demonstrate that mir-802 is broadly expressed in the intestinal epithelium of the
miR-802 regulates proliferation and intestinal epithelial cell turnover. Apart from their role in innate immunity and host-defense, Paneth cells also secrete factors such as WNT, NOTCH, EGF, BMP, that create the right microenvironment to sustain and modulate the stem cell niche in homeostatic and stress conditions. To explore whether miR-802 contributes to the regulation of proliferation, we analyzed the intestines of mice injected with thymidine analog 5′-bromo-2′-deoxyuridine (BrdU). Proliferation rates were increased in jejunal, but not ileal villi tips. To test this hypothesis, we performed BrdU-pulse labeling experiments by injecting mice with 

This finding was further supported by increased TUNEL staining at the tips of the villi in Vil-Cre mir-802/fl mice (Fig. 3h), furthermore, Cleaved Caspase 3 immunoblotting also demonstrated increased apoptosis in the jejunum of Vil-Cre mir-802/fl mice (Fig. 3h). Together, these findings raised the question of whether the increased turnover of enterocytes could lead to an impairment in their differentiation, as suggested by the reduced expression of glucose transporters, which is a hallmark of dedifferentiation in pancreatic β-cells. Expression analysis of the enterocyte-specific marker alkaline

jejenum and enriched in intestinal Paneth cells compared to enterocytes, where it regulates Paneth cell expansion.

To evaluate the impact of Paneth cell expansion on murine microbiota, the cecal and ileal microbiota composition of Vil-Cre mir-802/fl and mir-802/fl mice was analyzed by 16S rRNA gene amplicon sequencing. Overall, we measured a similar microbiota composition of the two mouse groups (Supplementary Fig. 2f). A Principal Component and Permutational MANOVA analysis revealed a cage effect and only a minor clustering by phenotype within a cage for the ileum samples (cage effect - R2 = 0.49, p = 0.001; phenotype effect - R2: 0.03, p = 0.56) (Supplementary Fig. 2g). Furthermore, no difference was found in the abundance of individual taxa by testing for differential abundance of zero-radius operational taxonomic units (zOTUs) between the animal groups, indicating that miR-802 has no major effect on the diversity and relative abundance of the microbiome (Supplementary Fig. 2h, i).

Dysregulation of the epithelial tight junction proteins have been linked to altered susceptibility to bacterial enteric pathogens. We measured transcript levels of the tight junction complex, including occludin, claudins and, zona occludens (Ocln, Cldn3, Tjp1), and found slightly increased transcript levels in the small intestine of Vil-Cre mir-802/fl mice, however, on a protein level, only CLDN was modestly upregulated (Supplementary Fig. 2j, k). Also, no functional changes in intestinal permeability were found when measuring fluorescein isothiocyanate-conjugated (FITC) dextran in serum following an oral gavage (Supplementary Fig. 2). However, protection against enteric salmonellosis has also been associated with increased intestinal defensin21 and lysozyme22 expression, and increased susceptibility has been reported in models with impaired Paneth cell function18,23 or after induction of Paneth cell degranulation24. To investigate if this association also holds in mice lacking mir-802, we orally administered high doses of virulent Salmonella Typhimurium to Vil-Cre mir-802/fl and control mir-802/fl mice. We measured significantly decreased recovery of viable S. Typhimurium from the mesenteric lymph nodes (mLN), with similar distribution in the spleen and cecal contents, indicating reduced bacterial dissemination and spread beyond the gut epithelium in the absence of intestinal miR-802 (Fig. 2g–j). Together, these findings indicate that mice with genetic ablation of mir-802 in the intestine may be more resistant to virulent S. Typhimurium than control mice, thereby suggesting a role for miR-802 in epithelial-derived defensins in the mammalian host defense.

miR-802 regulates glucose absorption in the small intestine. A Relative expression levels of miR-802 in indicated tissues (jejenum n = 4,4; liver n = 7,6; kidney n = 7,6; pancreatic islet n = 5,1; cortex n = 4,4). Data are representative of three independent experiments. B Relative miR-802 expression in female mice, measured by qRT-PCR, in indicated intestines sections (n = 6). Data are representative of two independent experiments C Oral glucose tolerance test (OGTT) of mir-802KO and control littermates (WT) (n = 8,9 per genotype). D, E OGTT (d) and IPGTT (e) in Vil-Cre mir-802/fl and mir-802/fl controls (n = 8,6 respectively for (d), n = 6 for (e)). F Serum GLP1 and insulin measurements of Vil-Cre mir-802/fl and control mice at the indicated time points after the oral glucose challenge (for (f) n = 7 for each genotype, for (g): wt n = 6 and KO n = 9). H Relative mRNA levels of Gip and Gpi1 in small intestines of Vil-Cre mir-802/fl and control mice (n = 10 per group). I GLP1 protein measurements of jejunum from Vil-Cre mir-802/fl and control mice (n = 7 per group, one experiment). j D-5C-Glucose levels in proximal jejenum (j), distal jejenum (k), and serum (l) following an oral 16C-glucose gavage (n = 8 for all groups, one experiment). M Heatmap representation of mRNA expression by RNA seq of glucose transporters in isolated jejunal enterocytes of Vil-Cre mir-802/fl and control mice shown as Log2FC (n = 3). N Relative mRNA expression, measured by qRT-PCR, of indicated glucose transporters in jejunal enterocytes of Vil-Cre mir-802/fl and indicated control mice (n = 9 per genotype). Data are representative of two independent experiments. O Immunoblot of GLUT2 in isolated enterocytes of the proximal jejenum of Vil-Cre mir-802/fl and control mice. γ-TUBULIN was used as a loading control. Quantification of densitometric analysis of signals is shown on the right. Each lane represents a lysate from a different mouse (n = 4 per genotype, one experiment). Data are plotted as mean ± SD. Significance was evaluated by two-tailed t test (j, l, o), by multiple two-tailed t-tests with Holm–Sidak method for multiple comparisons (a, h, i), one-way ANOVA with Tukey’s multiple comparison post-test (n), or Dunnet post test (b), and two-way ANOVA for repeated measures with Sidak’s multiple comparisons test (c–g).
Fig. 2 mir-802 regulates intestinal Paneth cell expansion. a FACS sorting of Paneth cells, intestinal stem cells (ISCs), and enterocytes from dissociated single cells of jejunal crypts and epithelium. ISCs were isolated as Lgr5-EGFP+, EPCAM+, CD24low/CD31-, Ter119-, CD45-, Efluoro780- and Paneth cells were sorted as CD24hi/SSChiLgr5-EGFP-, EPCAM+, CD31-, Ter119-, CD45-, Efluoro780- cells. An enriched enterocyte population was obtained by isolating remaining CD24low Lgr5-EGFP-, EPCAM+, CD31-, Ter119-, CD45-, Efluoro780- cells. Right: Relative miR-802 levels in Paneth cells, enterocytes, and stem cells as determined by qRT-PCR (n = 4 per group). Data representative of two independent experiments.

b Immunohistochemistry of proximal jejunal sections of mir-802fl/fl and Vil-Cre mir-802fl/fl mice stained with anti-LYZ antibody. Top panel: Scale bar: 50 µm. Right: Quantification of Paneth cell numbers/crypt (n = 5 per genotype). Representative images and analysis of two independent experiments.

c Percentage of sorted Paneth cells from single live cells from jejunal crypts of Vil-Cre mir-802fl/fl and control mice. Paneth cells were gated as CD24+, SSChi (n = 4 mice were pooled per genotype). Data representative of two independent experiments.

d Heatmap showing mRNA expression, measured by RNA seq, of secreted proteins in Vil-Cre mir-802fl/fl and control mice. Data are shown as Log2FC (n = 3).

e Relative expression of Lyz and defensins (e) and NF-κB and TNFa (f), measured by qRT-PCR, in isolated enterocytes of Vil-Cre mir-802fl/fl and control mice (n = 5.5 for (e) and n = 3.3 for (f)). Data representative of two independent experiments (e) and one experiment (f).

g-j Colonization experiment of 10-week-old pathogen-free mir-802fl/fl and Vil-Cre mir-802fl/fl mice that were gavaged with 10^8 CFU of stationary phase Salmonella Typhimurium (strain SL1344) cultures grown overnight. Bacterial levels in spleen, feces and mesenteric lymph nodes (mLNs) were determined by dilution plating of homogenized tissues. Colony formation unit per gram mLN (g), colony-forming units per mLN (h), colony-forming units per gram of cecal content (i), and colony formation unit per gram of spleen (j) (n = 10,11 for mir-802fl/fl and Vil-Cre mir-802fl/fl mice, respectively for (g, h, i) and n = 10 per genotype for (j), data are from two replicate experiments). Data are plotted as mean ± SD. Significance was evaluated by two-tailed t-test (b, e, f, one-way ANOVA with Tukey’s multiple comparisons test (a), or Mann–Whitney two-tailed test (g–j).
intestinal phosphatase (Alpi)\textsuperscript{28,29}, expressed in differentiated enterocytes but not in immature crypts, showed a significant reduction in Vil-Cre mir-802\textsuperscript{fl/fl} jejunal tissue compared to control jejunum (Fig. 3i). Furthermore, we measured increased levels of Prdm1, a transcriptional repressor that delays postnatal epithelial maturation\textsuperscript{30,31} (Fig. 3j). These data imply that mir-802 in the intestinal epithelium increases proliferation, accelerates epithelial cell turnover, perturbs absorptive enterocyte differentiation, and increases apoptosis.
Gene expression and miR-802 target identification in the intestinal epithelium. To address the molecular basis for the phenotypic alterations observed in mir-802 null mice, we performed RNA sequencing (RNA-Seq) analysis on isolated jejunal epithelial cells of Vil-Cre mir-802fl/fl and mir-802fl/fl control animals. We determined that 242 transcripts were downregulated with a cutoff of 0.5 log2 ratio (FDR < 0.01) in Vil-Cre mir-802fl/fl cells compared to controls, while 149 genes were upregulated using the same cutoff (Supplementary Fig. 4a). Gene Ontology (GO) enrichment analysis of differentially expressed genes (DEGs) identified proliferation and cell cycle as top upregulated biological process, which included genes of the Wnt–β-catenin pathway (i.e., Ccnd1, Myc), Il22ra1 signaling, known to stimulate Wnt expression and intestinal epithelial cell proliferation (Fig. 4a-c) [32]. In addition, we found strictly proliferation-dependent transcripts that are known positive regulators of cell proliferation (e.g., Mecom, Foxm1, Atm, E2F2, MKI67, Bmp2, Tbx3, Axin2) (Fig. 4c) [33-41]. We also identified increased levels of ROS-producing enzymes in Vil-Cre mir-802fl/fl mice, including Nox1, which harbors a 7mer-A1 mir-802 binding site in its 3′ UTR (Fig. 4d). Consequently, we measured increased levels of ROS and iNOS in the intestines of mice with mir-802 ablation (Fig. 4e, f). Since ROS-producing oxidases have been shown to act as modulators of Notch signaling in the intestine [42], we measured Notch expression in jejunal crypts and found them to be increased. Consistent with Notch upregulation we measured a modest increase of the target Hes1, however, Math1 was not repressed, suggesting that not all downstream components of the Notch pathway were activated in Vil-Cre mir-802fl/fl mice (Fig. 4g). Interestingly, RNA seq analysis also showed upregulation of several positive regulators of Paneth cell differentiation (e.g., Prdm1, Lgr4, Nox1, Il22ra1), maturation (Ppard), and crypt localization (EPHB2/3) in the intestine of Vil-Cre mir-802fl/fl. Lastly, we identified a total of 21 antioxidant genes with decreased expression levels in Vil-Cre mir-802fl/fl mice compared to controls (Supplementary Fig. 4b). They likely contribute to the increase in intestinal ROS, although none of these transcripts harbor miR-802 binding sites in their 3′ UTR. Together, these data suggest that loss of mir-802 activates several pathways regulating epithelial homeostasis in the intestine, including Wnt and Notch signaling and components of the ROS generating system.

We also identified many downregulated biological processes, amongst them metabolic pathways, in agreement with a total of 46 nutrient transporters that exhibited reduced expression in mice lacking mir-802 in the intestine (Supplementary Fig. 4c). This extensive downregulation of transporters further supports our finding that the intestinal epithelium of mir-802KO mice is not fully differentiated.

Direct target transcripts of miR-802 are expected to be derepressed and therefore upregulated in cells with genetic mir-802 ablation. Transcripts that carry miR-802 motifs showed significant upregulation in Vil-Cre mir-802fl/fl mice compared with transcripts that do not (P < 10-4, two-sided Mann–Whitney test), while no seed enrichment was observed for predicted targets of the ubiquitously expressed mir-16 (Fig. 4h, i). RNA-Seq analysis identified only 22 potential direct target transcripts of miR-802 that were regulated in intestinal cells of Vil-Cre mir-802fl/fl mice (Fig. 4j) Interestingly, we did not measure a significant upregulation of predicted miR-802 targets, such as Hnf1b, Gata4, and Msi1, which are known to affect Paneth cell and enterocyte differentiation [43-45]. Lastly, A1att, a previously reported target gene, was not derepressed in the liver and colon [46].

To further characterize the extent to which miR-802 is capable of repressing the endogenous transcripts of the top predicted targets, we overexpressed miR-802 at different MOIs and measured endogenous target transcript levels [7]. Surprisingly, in contrast to a previous report [8], Hnf1b showed little repression, whereas the transmembrane emp24 domain-containing protein-9 (Tmed9) transcript was by far the most sensitive to dose-dependent inhibition by mir-802 (Supplementary Fig. 4d). Tmed9 harbors two evolutionary-conserved 8-mer seeds (Fig. 4k), and is targeted in TargetScan [7,8]. More recently, the same group showed that miR-802 targets could influence the Wnt signaling network and therefore might explain the phenotype of Vil-Cre mir-802fl/fl mice. Indeed, we identified and independently validated the derepression of six conserved targets in isolated crypts of Vil-Cre mir-802fl/fl compared to mir-802fl/fl mice. They included Tmed9, the Wnt...
receptor Fzd5, two members of the β-catenin destruction complex (Axin2 and Apc), Tcf4 a downstream target of Wnt signaling, and the NADPH oxidase1 (Nox1) (Fig. 4l). To test whether the predicted miR-802 target sites in the 3′ UTR of Tmed9, Fzd5, Axin2, Apc, Tcf4, and Nox1 mRNA were responsible for their derepression in the absence of miR-802, we cloned their putative 3′ UTRs downstream of a luciferase reporter gene and co-transfected this vector into HEK293 cells, which do not express miR-802 and are sensitive to changes in reporter activity, along with a vector driving the expression of miR-802 or a control vector (Fig. 4m, Supplementary Fig. 4e). Luciferase activity of cells with forced miR-802 overexpression was decreased by ≈80%, 20%, 30%, 20%, 50%, and 30%, respectively, relative to that of cells that were co-transfected with control vectors. Point
mutations in the seed region of the mir-802 target site (mut) abolished the repression of mir-802 on luciferase activity (Fig. 4m). These data show that Tmed9, Fzd5, Axin2, Apc, Tcf4, and Nolx1 are direct targets of mir-802 in epithelial cells and that their repression is mediated by mir-802 target sites in their respective 3′UTRs. In conclusion, we identified and validated six mir-802 targets related to Wnt signaling with a possible role in the regulation of intestinal stem cell proliferation.

**Tmed9 is a mir-802 target that modulates Wnt/β-catenin signaling.** To further establish that Tmed9 is a mir-802 target in the intestine we generated mouse small intestine-derived organoids and overexpressed mir-802 using recombinant adenovirus. Results showed that Tmed9 is repressed upon mir-802 overexpression (Fig. 5a). In addition, Tmed9 protein levels, determined by Western blotting, were derepressed in intestines of *Vil-Cre mir-802fl/fl* and in jejunal *Vil-Cre mir-802fl/fl* derived organoids compared to respective wild-type controls (Fig. 5b, Supplementary Fig. 5a). Moreover, Tmed9 transcript levels, measured by qRT-PCR, were increased in FACS-sorted Paneth cells of *Vil-Cre mir-802fl/fl* mice compared to *mir-802fl/fl* controls (Fig. 5c). These results confirm Tmed9 as a mir-802 target in the small intestine and Paneth cells.

Paneth cells are the major epithelial WNT producers within the intestinal crypts and a previous study has shown that Mir122, measured by qRT-PCR, in isolated crypts from upper jejunum (n = 4 per group, one experiment). c Heatmap of transcript expression from RNAseq data of positive regulators of the cell cycle (GO 0007126) shown as Log2FC (n = 3). d Heatmap of transcript expression from RNAseq data of ROS-producing genes (top) and Notch2 and downstream targets (bottom) shown as Log2FC (n = 3). e, f ROS (H2O2) (e) and iNOS (f) production in the upper jejunum of *Vil-Cre mir-802fl/fl* and *mir-802fl/fl* mice (n = 11 per genotype for ROS, two independent experiments) (n = 8, 7 per genotype for iNOS, respectively, one experiment). g Relative expression measured by qRT-PCR of Notch targets Hes1 and Math1 in isolated enterocytes of upper jejunum (n = 8 per genotype, one experiment). h, i Global mir-802 target gene regulation from RNAseq (n = 3 per group). Cumulative density plots of RNA-seq data for mir-802 (h) and mir-16 targets (i) (negative control), grouped by context score (ccs) based on Target Scan 7.1. j Heatmap from RNAseq data of 22 differentially expressed target genes of mir-802 shown as Log2FC (n = 3). k Gene organization and evolutionary conservation of mir-802 target sites in 3′UTR of Tmed9. l Validation of indicated mir-802 target gene expression by qRT-PCR in isolated crypts of upper jejunum from *Vil-Cre mir-802fl/fl* and *mir-802fl/fl* mice. n = 9,9,3,3,3,3 for WT crypts, 7,12,4,3,3,3 for KO crypts, respectively, one experiment. m Dual-luciferase assays for mir-802 targets (Tmed9, Fzd5, Axin2, Apc, Tcf4, Nolx1) in HEK293T transfected with plasmid-pmirGLO-WT 3′UTR (WT) or plasmid-pmirGLO-mutant 3′UTR, and miRNA mimics for mir-802 or control (Ctrl). Firefly luciferase activity was normalized to Renilla luciferase activity and expressed relative to control. n = 3,3,5,6,4,4 for WT + Ctrl, 3,3,3,6,6,4 for WT + mir-802, 3,4,4,4,4,4 for Mut + Ctrl and 2,3,3,6,4,4 for Mut + mir-802, one experiment. Data are plotted as mean ± SD. Significance was evaluated by a two-tailed t-test (b, e–g, l), or one-way ANOVA followed by Tukey’s multiple comparison test (m).
We next investigated whether Tmed9 derepression in the intestinal epithelium of Vil-Cre Tmed9Kmut/mut mice would partially phenocopy Vil-Cre mir-802fl/fl animals. No metabolic abnormalities were observed in response to an oral glucose challenge (Fig. 6d). Furthermore, RNA seq analysis of intestinal epithelium from Vil-Cre Tmed9Kmut/mut and control Tmed9Kmut/mut mice did not show changes in the expression of solute carrier transporters or Alpi (Supplementary Fig. 6e), indicating that enterocyte differentiation was not affected by Tmed9 overexpression. Nonetheless, expression of defensins and Lyz was increased in Vil-Cre Tmed9Kmut/mut animals (Fig. 6e, f), in line with increased Tmed9 levels in sorted Paneth cells (Fig. 6g). To obtain further evidence that TMED9 regulates the secretion of antimicrobial peptides, we perturbed TMED9 levels in C2BBe1 cells using adenoviral overexpression or RNAi and compared LYZ concentrations in the supernatant. Overexpression of TMED9 increased LYZ secretion in the supernatant, whereas silencing of TMED9 resulted in borderline decreased LYZ expression (Fig. 6h, i). In addition, overexpression of miR-802 reduced LYZ secretion (Fig. 6j). However, Vil-Cre Tmed9Kmut/mut mice were not protected from Salmonella invasion compared to Tmed9Kmut/mut mice (Supplementary Fig. 6f), most likely due to a smaller increase in defensin expression compared to Vil-Cre mir-802fl/fl mice (Fig. 6e). Similarly, Paneth cell numbers were similar (Supplementary Fig. 6g) and epithelial proliferation and apoptosis...
Fig. 5 TMED9 is a mir-802 target that modulates Wnt/β-catenin signaling, a qRT-PCR of Tmed9 (right) and mir-802 (left) of mouse small intestine-derived organoids infected with mir-802 adenovirus compared to control adenovirus (n = 4 per group, two independent experiments). b Immunoblot of TMED9 protein in lysates of upper jejunum from Vil-Cre mir-802fl/fl and mir-802+/+ control mice. β-ACTIN was used as a loading control. Quantitative analysis of densitometric measurements is shown on the right. Each lane represents a lysate from a different mouse (n = 3 per genotype). Representative data of two independent experiments. c Tmed9 transcript levels, measured by qRT-PCR, in FACS-sorted Paneth cells of Vil-Cre mir-802fl/fl mice compared to mir-802+/+ controls (one independent experiment, each dot represents 3 pooled mice, n = 3,2 per genotype)). d Immunoblot analysis of supernatant from C2BBe1 cells expressing FLAG-tagged WNT3a that were infected with either Ad-miR-802 or control Ad-GFP. The image of the Ponceau stained gel is shown as a loading control. γTUBULIN of cell lysates was used as an additional loading control. Quantitative analysis of densitometric measurements is shown on the right (n = 3 per group, two independent experiments). e Immunoblot analysis of β-CATENIN from isolated epithelial cells of upper jejunum of Vil-Cre mir-802fl/fl and mir-802+/+ mice. Quantitative analysis of densitometric measurements is shown on the right. Each lane represents a lysate from a different mouse (n = 3 per genotype, one experiment). f Cell fractionation followed by immunoblotting of β-CATENIN from isolated crypts. H3 was used as a nuclear marker. Each lane represents a lysate from a different mouse (n = 2 per genotype, two independent experiments). g, h Immunoblot analysis of β-CATENIN in C2BBe1 cells infected with Ad-miR-802 (g) or Ad-Tmed9 (h) and control Ad-GFP adenoviruses. β-ACTIN was used as a loading control. Quantitative analysis of densitometric measurements is shown on the right (n = 3 per group, one experiment). i, j C2BBe1 cells were transduced with Ad-mir-802 (i) or Ad-Tmed9 (j) or control Ad-GFP virus, and transfected after with TOPFlash or FOPFlash plasmids together with Renilla luciferase (internal control). After 36 h cells were lysed and assayed using the dual-luciferase reporter system (n = 8,4,3,4 for (i) and n = 4 for (j) (one experiment)). k C2BBe1 cells were transfected with siTmed9 or control siRNAs and TOPFlash or FOPFlash plasmids together with Renilla luciferase (internal control) (n = 6,6,4,4, one experiment). Data are plotted as mean ± SD. Statistical significance was evaluated by unpaired two-tailed t-test (a-e, g, h) or by one-way ANOVA followed by Tukey’s multiple comparison test (f-k).

Discussion

Our study has demonstrated that mir-802 plays an important role in epithelial intestinal homeostasis, which is supported by high expression levels of mir-802 in Paneth cells and enterocytes as well as altered phenotypes in these cell types. Interestingly, during evolution mir-802 first emerged in bony fish during the Silurian period 430 million years ago, when the short elasmobranch digestive system expanded into an intestine, which is absent in non-teleost fish38,59. Many bony fish also contain Paneth cells and enteroendocrine cells in their crypts. Furthermore, at least one of the Tmed9 target sites in the 3'UTR is present in bony fish, whereas the mir-802 sites are absent in Wnt signaling genes Zsd5, Axin2, Apc, and Tcf4 (Fig. 4k, Supplementary Fig. 4j).

Our study in mir-802-deficient mice uncovered a pleiotropic phenotype manifested by increased proliferation, increased Paneth cell numbers/function, and partly dedifferentiated enterocytes (Fig. 6k). There is increasing evidence that an intricate functional relationship exists between Wnt and Notch signaling during the assignment of particular cell fates, for stem cell maintenance, and a proper balance of differentiation between secretory and absorptive cell lineages15. Genetic studies in mice have shown that Wnt signals are a major driver of a stem-cell/progenitor gene program and also confer competence for secretory fate decision, most notably by promoting a Paneth-cell maturation program16,30–52,60. On the other hand, Notch signaling negatively regulates secretory cell differentiation through repression of Math1/Atoh161, whereas in the absence of Notch, stem cells preferentially generate secretory cells at the expense of absorptive cells62. Notch activity is required for maintaining the correct balance of Wnt signaling in the crypt, which allows for simultaneous maintenance of ISCs, proliferation, and differentiation63. The increased proliferation rate and Paneth cell numbers in the jejunum of mice lacking mir-802 is likely mediated by increased secretion of Wnt in the intestinal crypts, and by the activation of their downstream effectors LGR5 and TCF464,65. Our finding that Notch expression is elevated in the intestines of mice lacking mir-802 is seemingly contradictory, and the fact that we could measure only small or no responses in the classical Notch targets Hes1 and Math1 is indicative that the Notch pathway is not dominant. We also show that mir-802 is a negative regulator of Wnt signaling, by repressing Tmed9 expression in Paneth cells, thereby inhibiting Wnt (and defensin) secretion. In addition to Wnt inhibition, mir-802 also directly represses components of the Wnt signaling cascade, most notably Fzd5 and downstream target Tcf4. However, it is worth noting that mir-802 also targets Axin2 and Apc, two negative regulators of Wnt signaling, and components of the β-catenin degradation complex66,67. In addition, we also observed a marked overexpression of the Il22ra1 receptor complex that is essential for Wnt secretion, epithelial cell growth, and Paneth cell function, including the production of antimicrobial peptides32,68. Together, these findings are in line with the role of miRNAs in buffering pathway activities at different levels by simultaneously dampening the expression of both positive and negative regulators, thereby avoiding runaway pathway activation and preventing stochastic fluctuations in signaling69.

In addition to the complex and intricate regulation of major signaling pathways regulating intestinal epithelial homeostasis, mir-802 also dampens the expression of several ROS-producing genes. Indeed, we noted that ablation of mir-802 results in increased ROS and iNOS levels. Emerging evidence in the cardiac system and intestine indicates that Notch and Wnt/β-catenin pathways are influenced by Nox-derived ROS42,70, suggesting that this pathway may also contribute to the observed mir-802KO phenotype.

Our study identified mir-802 and its target Tmed9 as a regulator of Paneth cell number and antimicrobial peptide expression. We show that increased TEMD9 levels not only promote WNT secretion but also enhance the production of LYZ and defensins from Paneth cells. The strongest evidence for Tmed9’s role in Paneth cell secretion comes from in vivo studies in mutant Tmed9 KO mice that exhibit a selective upregulation of Tmed9 and identified increased Lyz and defensin levels in the absence of increased Paneth cell numbers. Paneth cells are responsible for the host defense of the intestinal tract by secreting antimicrobial peptides as a first line of defense. Enhanced Paneth cell function in mir-802 knockout mice was demonstrated in S. Typhimurium
Fig. 6 Mutation of miR-802 binding sites in Tmed9 is sufficient to phenocopy the Vil-Cre mir-802fl/fl Paneth cell phenotype. 

a) Fraction (%) of Tmed9 mutant allele expression on Tmed9 expression in the intestinal epithelium of Vil-Cre Tmed9KImut/mut, Vil-Cre Tmed9KImut/mut, and control Tmed9KImut/mut mice. Box plot defined by minima (0, 58.43, 95.46), median (1.377, 60.55, 96.21), maxima (2.5, 61.39, 98.98), 25% percentile (0,58.43, 95.6), 75% percentile (2.5, 61.39, 98.33), range (2.5, 2.957, 3.520), mean (1.292, 60.12, 96.71) for genotypes Tmed9KImut/mut, Vil-Cre Tmed9KIwt/mut, Vil-Cre Tmed9KImut/mut respectively with line shown at mean (n=3, 3, 4 respectively per genotype).

b) Relative mRNA expression, measured by qRT-PCR, of Tmed9 in the isolated intestinal epithelium of Vil-Cre Tmed9KImut/mut, Vil-Cre Tmed9KImut/mut, and control Tmed9KImut/mut mice (n=3,3,4 for each genotype respectively). Data are representative of three independent experiments.

c) Immunoblot analysis of TMED9 in the jejunum of Vil-Cre Tmed9KImut/mut, Vil-Cre Tmed9KIwt/mut, and control Tmed9KImut/mut mice. β-ACTIN was used as a loading control. Quantitative densitometric analysis is shown on the right. Each lane represents a lysate from a different mouse (n=3 per genotype). Data are representative of two independent experiments.

d) Oral glucose tolerance test of Vil-Cre Tmed9KImut/mut and control Tmed9KImut/mut mice (n=7 for each group, two independent experiments).

e) Relative mRNA expression levels, measured by qRT-PCR, of antimicrobial peptides in jejunum of Vil-Cre Tmed9KImut/mut, Vil-Cre Tmed9KImut/mut, and control Tmed9KImut/mut mice. β-ACTIN was used as a loading control. Quantitative densitometric analysis is shown on the right. Each lane represents a lysate from a different mouse (n=4 per genotype, two independent experiments).

f) Immunoblot analysis of LYZ in isolated enterocytes from upper jejunum of Vil-Cre Tmed9KImut/mut and Tmed9KImut/mut littermates. The quantification of densitometric analysis is shown on the right. Each lane represents a lysate from a different mouse (n=4 per genotype, two independent experiments). g) Tmed9 transcript levels, measured by qRT-PCR, in FACS-sorted Paneth cells of Vil-Cre Tmed9KImut/mut mice compared to Tmed9KImut/mut littermates (n=4 for each genotype, one experiment).

h) Immunoblot analysis of LYZ in C2BBe1 cells that were infected with Ad-Tmed9 (h) or transfected with siRNAs targeting Tmed9 or control siRNAs (i). β-ACTIN was used as a loading control. Quantitative densitometric analysis is shown on the right (n=3 per group). Data are representative of two independent experiments.

i) Immunoblot analysis of LYZ in C2BBe1 cells infected with Ad-miR-802 and control (Ad-GFP) adenovirus. β-ACTIN was used as a loading control. Quantitative densitometric analysis is shown on the right (n=3 per group). Data are representative of two independent experiments. Data are plotted as mean ± SD. Statistical significance was evaluated by descriptive statistics (a) unpaired two-tailed t-tests (f-j), by one-way ANOVA followed by Tukey’s multiple comparison test (b, c, e), or two-way ANOVA for repeated measures with Sidak’s multiple comparisons test (d).
infection experiments, showing partial protection in Vil-Cre mir-802fl/fl mice. These data are consistent with previous studies in which protection against Salmonella infection was demonstrated in transgenic mice overexpressing a single human defensin (HD5) or lysozyme21,22, or following treatment of recombinant anti-microbial human β-defensin hBD-1 and hBD-2.43. However, we stress that the Salmonella findings merely provide associations and further studies and models are warranted to establish a cause-and-effect mechanistic relationship linking PC changes in mir-802KO mice to the resistance to microbial infection. Additional mechanisms may also be responsible for the heightened anti-microbial defense in mice with mir-802 ablation, including the upregulation of Il22ra1 and Reg3y, consistent with studies showing that Il22 activates Reg3y and that this pathway is protective against mucosal Salmonella infection in vivo.20,72. However, the precise role of mir-802 in the antimicrobial defense warrants further and more detailed investigations in gram-positive and negative invasion models.

Several studies have demonstrated the ability of intestinal commensals to induce mucosal innate immune effectors, including Reg3y72 and angiogenin73. Studies investigating the effect of immune factors on microbiome composition, however, are more limited. A study in zebrafish has demonstrated that the host plays an important role in selecting its particular biota74. Furthermore, studies in mice with defense deficiency (Mmp7fl/fl) or surplus (Hd5fl/+/-) have shown a defense-dependent reciprocal shift in the dominant bacterial species of the small intestine, without changes in total bacterial numbers.75. Our findings did not identify significant differences in microbiota composition of cecal content as well as ileal content from mir-802 null mice. We believe this can be best explained by the topology of mir-802 expression and action, which is restricted to the upper small intestine where microbiota mass is small. Furthermore, our microbiota studies also indicate that the phenotypic effects in mir-802 knockout mice are largely unrelated to the microbiota.

We investigated the mir-802–Tmed9 axis using a knock-in model in which the mir-802 sites in the 3’UTR of Tmed9 are selectively mutated, leading to selective derepression of Tmed9 in the intestine. These mice showed a partial Paneth cell phenocopy and increased LYZ and defensin expression. However, the antimicrobial phenotype was more pronounced in Vil-Cre mir-802fl/fl mice, indicating that this pathway is also regulated by additional miR-802 targets. This may also explain why stem cell proliferation and Paneth cell numbers were not changed in Vil-Cre Tmed9KImut/mut mice and why no protective effect against Salmonella infection was observed. In addition, increased ROS was only seen in Vil-Cre mir-802fl/fl mice, suggesting that ROS metabolism is regulated by many direct (Noxl) and indirect targets (antioxidant genes), independent of Tmed9.

Lastly, a previous study reported that mir-802 regulates glucose homeostasis in the liver through a mechanism involving the repression of the transcription factor Hnf1β. They were unable to detect differences in Hnf1β and glucose levels or insulin tolerance of mice with liver-specific deletion of mir-802 under Chow or high-fat diet conditions. Our data rather indicates that mir-802 influences glucose metabolism through regulating glucose uptake and intestinal homeostasis.

In conclusion, we have identified and genetically validated a previously unknown regulatory miRNA network that is responsible for maintaining normal intestinal epithelial homeostasis and linking mir-802, TME9, and WNT signaling. Considering the importance of this pathway in inflammatory bowel disease (IBD) and cancer it will be important to investigate the pathophysiological role of mir-802 in initiation, remission, and relapse of IBD as well as in the context of epithelial tumor progression and formation.

**Methods**

**Animal husbandry and mouse strains.** All mice were on a pure C57BL/6N background. Mice were housed in a pathogen-free animal facility at the Institute of Molecular Health Sciences at ETH Zurich. The animals were maintained in a temperature- and humidity-controlled room on a 12 h light/12 h dark cycle (lights on from 6 am to 6 pm). Mice were fed a standard laboratory chow diet and had access to water ad libitum. Mice were 6–10 weeks of age unless indicated otherwise in the figures and text. All ethical regulations were complied with and all animal experiments were approved by the Kantonale Veterinärämter Zürich. Vil-Cre mice (B6.129-Tg(Vil-cre)997Gum/l), were purchased from Jackson Laboratories and Lgr5-Cre (B6.129P2Ztg5tm1(creERT2)Elt/J) mice were a gift from H. Clevers.

**Generation of conditional mir-802 knockout mice.** mir-802 is an intergenic miRNA located on mouse chromosome 16 (chr16: 93369720–93369816+), and it was targeted through the introduction of loxP sites (34 bps) at positions chr16: 93369116 (575 bp upstream of mir-802) and chr16: 93370283 (468 bp downstream of mir-802). The 15.9 kb length targeting construct harboring mmu-miR-802 (Amnie/1480ABC A08) was generated by PCR cloning using a BAC template. loxP sites were placed 608 bps upstream and 2287 bps downstream of mir-802. The neomycin cassette with FRT sites was inserted right before the second loxP site 1046 bp downstream of mir-802. All sequences were validated by DNA sequencing. The targeting vector was electroporated into C57BL/6 embryonic stem (ES) cells and single clones, selected for neomycin resistance, were picked and screened for homologous recombination by Southern blotting. Subsequently, correctly targeted ES cells were injected into blastocysts and transplanted into pseudopregnant females. The resulting chimeric offspring were bred to FLP-deleter mice for excision of the neomycin excision cassette and genotyped by Southern blotting. For tissue-specific mir-802 ablation, the following strains were utilized: Vil-Cre (for intestinal epithelium-specific deletion), and Lgr5-Cre to genetically mark the Lgr5 positive stem cell population.

**Generation of conditional Tmed9 3 UTR mutant knock-in (Tmed9Ktmut) mice.** The BAC clones RP23-62D23 and RP23-216P14 from the C57BL/6J library containing the mouse Tmed9 gene (NCBI Reference Sequence: NM_026211.3) were used as a template to generate the homology arms by high fidelity PCR and sequencing. In the targeting vector, the Neo cassette was flanked by Rox sites76. A targeting vector was generated in which the mutant exon 5, flanked by lox2272 sites77 and containing two GTACTGG to GTATGTG mutations, was cloned downstream of the wild-type exon 5, containing the TAG stop codon and 3’ UTR, in the reverse orientation. Wild-type and mutant exon 5 were flanked with loxP sites. After confirming correctly targeted ES clones via Southern Blotting, some clones were selected for blastocyst microinjection, followed by chimera production. Founders were confirmed as germ-line-transmitted via cross-breeding with wild-type mice. For intestinal epithelium-specific deletion the Tmed9Ktmut/mut mice were crossed with the Villin-Cre (B6.129-Tg(Vil-cre)997Gum/l).

**Metabolic experiments.** For intraperitoneal glucose tolerance tests (iPGTT), female or male mice were fasted for 6 h and then injected with 2 g/kg body weight D-glucose in PBS. In oral glucose tolerance tests (oGTT), glucose was orally administered by gavage. Blood glucose was measured using a Contour glucometer (Bayer) at specific time points. For insulin tolerance tests, mice were intraperitoneally injected with 0.4 U/kg insulin solution (Insulin solution human, Sigma Aldrich) and blood glucose was measured at the time points indicated in figures.
In vivo GLP-1 secretion and GLP-1 content assays. In GLP-1 secretion assays, female mice were injected intraperitoneally with dipheretia peptidase-4 (DPP-4) inhibitor Sitagliptin (Merck, 5 mg/kg) at t = 0 and after 30 min, with D-glucose (Sigma Aldrich 3 g/kg). Blood was sampled 5 min thereafter and added to 5 μl NacProtein (Sigma, 5 mg/ml), 2 μl EDTA (0.5 M) and, 3 μl DPP-IV Inhibitor (Millipore) on ice. The serum was collected and GLP-1 content was measured with GLP-1 ELISA (Merck).

Luciferase assays. HEK-293 cells were cultured in 96-well plates and transfected with 20 ng pmirGLO reporters together with miRNA mimics (40 nM) or Adeno- virus. miRNA-Clariﬁed media were harvested 48 h after transfection and assayed for GLP-1 luciferase Reporter Assay System (Promega). Results were normalized to the Renilla luciferase control contained in pmirGLO and expressed relative to the average value of the controls.

Plasmids and recombinant adenosviruses. A mouse miR-802-expressing vector was generated by PCR ampliﬁcation of a region consisting of the miR-802 stem-loop, with an additional 142 and 156 bp of genomic sequence at the 5’ and 3’ ends, respectively. Fragments were cloned into pFaMHi and NcoII sites of pVQACMV_K- NPA for adenovirus production (Viraquest). For generation of VQAD Tmd96-GFP (Ad-Tmd96), human TMD96 cDNA was subcloned from a plasmid (Origene, MC200132, cDNA clone MG: 7860) into pVQACMV_K, NPA by restriction digestion with KpnI and XhoI (1429 bp insert). All adenoviruses harbored a GFP gene with an independent (RSV) promoter as a control for infection efﬁciency. Ad- Ctrl was based on the same vector backbone (including GFP) but lacked the transgenes. Wnt3a-FLAG plasmid was obtained from Orgene, and M50 Super 8x TOPFlash (WT, #12456) and M51 Super 8x TOPFlash (TOPFlash mutant, #12457) plasmids were obtained from Addgene.

Enterocyte and Crypt isolation. Enterocytes were isolated from the duodenum (2 cm), upper jejunum (referred to the proximal half of jejunum), lower jejunum (distal half of the jejunum), and ileum (2 cm segment before cecum) as well as the colon of female 6–8 week old mice using a solution of 5 mM EDTA/PBS, supplemented with Complete protease inhibitor mixture (Roche) (40 μg/ml) for 30 min at 4°C. The cell suspension was then washed in ice-cold buffer (119 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl2, 1.2 mM MgSO4, 1.2 mM KH2PO4, 25 mM NaHCO3, pH 7.4). Tissues were then solubilized in Soluene (Perkin Elmer) and radioactivity was measured in a liquid scintillation counter. For intestinal glucose uptake analysis, the whole small intestine was longitudinally opened and washed in ice-cold Krebs buffer for 10 min at 4°C. The tissue was then solubilized in Soluene (Perkin Elmer) and radioactivity was measured in a liquid scintillation counter. Background counts from non-radioactive control samples were subtracted from each sample.

In vivo glucose-stimulated insulin secretion assay. Female mice were orally administered with 2 g/kg D-glucose. 15 and 30 min later, blood samples were collected and plasma insulin concentrations were determined by rat insulin enzyme-linked immunosorbent assay (ALPCO, Salem, NH).

Immunoblotting. Lysates of mouse tissues or cells were homogenized in RIPA Lysis buffer (150 mM NaCl, 50 mM TrisHCl pH 8, 5 mM EDTA pH 8, 5 mM EGTA pH 8, 1% NP-40, 0.5% Sodium deoxycholate, 0.1% SDS (Sigma-Aldrich) containing EDTA-free protease inhibitor cocktail (Roche Diagnostics) (1 tablet/50 ml). After centrifugation, pellets were homogenized by scraping and sonication, while tissues were disrupted using stainless steel beads and a tissue-Lyser II (Qiagen). For analysis of secreted proteins, cell media were collected and concentrated using Amicon centrifugal filters. Supernatants were transferred to a new tube prior to determining the protein concentration using the bicinchonic acid (BCA) kit (Sigma-Aldrich). After adding the ammonium bicarbonate buffer, lysates were boiled for 5 min at 85 °C and proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and transferred onto nitrocellulose membranes by electroblotting in a wet chamber (Bio-Rad Laboratories). The membranes were stained with Ponceau S and blocked with 5% non-fat dry milk TBS-0.1%Tween (Sigma-Aldrich). The blots were incubated with primary antibodies overnight at 4 °C and developed using horseradish peroxidase-conjugated secondary antibodies (Calbiochem) followed by chemi-luminescent detection with a Fujifilm analyzer (LAS-4000). Uncropped blots are found in the Source data file.

Antibodies. The antibodies used for immunoblotting are listed in Supplementary Table 1.

Flow cytometry and isolation of ISC, Paneth cells, and CD24+CD49f enriched stem cell fraction. Crypts and enterocytes were isolated from the upper jejunum (6 cm) of the small intestine of female mice as described previously in the methods. ISC and Paneth cells were isolated from the small intestine by flow cytometry following the protocol previously published81. In short, the crypt suspensions were centrifuged for 5 min at 250 × g (4 °C or room temperature). The pellets were resuspended in 1.0 ml of undiluted TrypLE Express (Invitrogen) + 120 μl of DNase I (10 U/ml) and transferred to 15 ml Falcon tubes. Samples were incubated at 32 °C in a water bath for 2 min. Then, 12 ml cold DMEM (Invitrogen) were added to the samples and triturated twice, followed by centrifugation at 250 × g at 4 °C. Both enterocytes and crypt cells were then stained in cell staining buffer (Biolegend) with an antibody cocktail containing CD45-PE (eBioscience, 30-F11), CD31-PE (Biolegend, Mec13.3), Ter119-PE (Biolegend, Ter119), CD24-Pacific Blue (Biolegend, M1/69) and EPCAM-APC (17-5791-82, ThermoFisher Scientific) for 30 min at 4°C in the dark. After that cells were washed twice with PBS and stained in a cell staining Buffer with EFlour-780 Viability dye (ThermoFisher Scientific, 65-0865-14) for 15 min. After washing cells twice, the pellets were dissolved in 2 ml FACS Buffer (2% FBS-PBS) and samples were filtered through 40 μm mesh (BD Falcon) and were further proceeded for sorting. Intestinal stem cells were isolated as Lgr5+Egfp+ EpCAM+ CD24low/CD31− Ter119− CD45− EFlour780− 81. Paneth cells were isolated as CD24hi/CD49fLgr5-Egfp− EpCAM+ CD24+ Ter119+ CD45− EFlour780+. Enriched enteroendocrine population was isolated as CD24+ Lgr5-EgFP EpCAM+ CD24+ Ter119+ CD45− EFlour780− with a BD FACS Aria II SORP cell sorter. Enriched stem cell fraction was sorted as live single CD24− CD24+ as previously shown32. Cells were incubated with both CD24-PE (BD 553106) and CD45-FITC (BD 553090) antibodies at 0.25 μg per 1 × 10^6 cells in 100 μl PBS/1% BSA, for 15 min, RT, and washed with PBS/1% BSA. PicPure RNA isolation kit was used for RNA isolation.
Illumina RNA sequencing and analysis. RNA was isolated from enterocytes of 14-week-old female mice using Trizol (Sigma), followed by RNasey (Qiagen) column purification with on-column DNase (Sigma) digestion. The quality of isolated RNA was determined with a Qubit (1.0) Fluorometer (Life Technologies) and a Bioanalyzer 2100 (Agilent). The TruSeq RNA Sample Prep Kit v2 (Illumina) was used according to the manufacturer protocol. Briefly, total RNA samples (100–1000 ng) were poly-A enriched and then reverse transcribed into double-strand cDNA. The cDNA samples were fragmented and repaired and polyadenylated before ligation of TruSeq adapters containing the index sequence for multiplex sequencing. Multiplexing fragments containing TruSeq adapters on both ends were selectively amplified with PCR. The quality and quantity of the enriched libraries were validated using Qubit (1.0) Fluorometer and the Caliper GX LabChip GX (Caliper Life Sciences Inc.). The product is a smear with an average fragment size of ~260 bp. The libraries were normalized to 10 nM in Tris-Cl 10 mM, pH 8.5, with 0.1% Tween 20. RNA-Seq read quality were checked with FastQC (Babraham Bioinformatics), which computes various quality metrics for raw reads. Reads were aligned to the genome and transcriptome with TopHat v 1.3.3 (https://ccb.jhu.edu/software/tophat/manual.shtml). Before mapping, the low-quality ends of the reads were clipped (3 bases from the read start and 10 bases from the read end). TopHat was run with default options. The fragment length parameter was set to 100 bases, with an SD of 100 bases. Based on these alignments, the distribution of the reads across genomic features was analyzed. For seed enrichment analysis, only genes with FPKM above 1.0 were considered as targets. For cumulative distribution function calculations, log2 fold-change values were corrected for 3′ UTR length biases. To detect differentially expressed genes we applied a count-based negative binomial model implemented in the software package EdgeR (R version: 3.3.0, EdgeR version: 3.14.0) [EdgeR]. The differential expression was assessed using a glm adapted for over-dispersed data. Genes showing altered expression with a p-value < 0.05 were considered as differentially expressed. Samples were sequenced at the Functional Genomics Center, Zürich (FGGCZ) using the Illumina HiSeq 2500.

DNA extraction for 165 rRNA gene sequencing. Cecal and ileal tissue were collected from co-housed wildtype (mir-802 fl/fl and Ccl-6 mir-802 fl/fl) mice fed conventional mouse chow. Isolated ileal and cecal tissue was immediately flash-frozen and stored at −80°C. RNA was extracted using the AllPrep DNA/RNA Kit (Qiagen) with the following changes in the disruption and homogenization steps: 600 mL of RLT buffer and two 3 mm metal beads were added to the tube containing the cecal content and bead-beaten at 10 Hz for 2 min using the mixer mill Retch MM400. To separate fibers from bacteria, samples were centrifuged at 1500 x g for 2 min. Supernatant containing the bacteria was transferred to a tube containing 0.9 mg of 0.1 mm zirconia beads (OPS Diagnostics) and samples were homogenized twice at 30 Hz for 3 min with 5 min incubation between each homogenization run. Before the transfer to DNA-binding column, the samples were centrifuged at full speed for 3 min to pellet the cell debris. The supernatants were added onto the DNA binding columns and DNA was eluted in 100 μL elution buffer (EB). A water sample as negative control and a sample with DNA from E. coli DH5α as positive control were processed in the same way as the cecal samples.

Library preparation and 16S rRNA gene sequencing. The library was produced with a two-step PCR approach using the amplicon deep sequencing primers from Microsynth. The first-step PCR used the locus-specific degenerative primers 515F (5′- GTGYCAGCMCCGGCGCTTA-3′) and 806R (5′-GGACTACNVGGGTWTATCA-3′) targeting the variable region 4[40,44]. The PCR reaction was performed using Q5 High-Fidelity DNA polymerase (BioConcept, New England Biolabs) under the following cycling conditions: 1. initial denaturation, 95°C for 4 min; 2. denaturation, 95°C, for 30 s; 3. annealing, 56°C for 30 s, 4. extension, 72°C, for 30 s; 5. final extension, 72°C, for 4 min. Cycles 2–4 were repeated eight times for the first PCR reaction and twenty times for the second PCR reaction. After each PCR reaction, the PCR products were cleaned from oligos and nucleotides using 0.8 x reaction volumes of CleanNGS magnetic beads (LABGENE SCIENTIFIC SA). The cleaned PCR product was eluted in 14 μL of EB and used in a second PCR reaction with barcoded primers or for producing multiple amplifyers. The quantity of amplified amplicons was determined using a Quantifluor儒meter (Thermo Fisher Scientific). The quality of the amplicons was verified using a fragment analyzer (Advanced Analytical). The length of the amplicons after barcoding was ~450 bp. PCR products were adjusted to a final concentration of 60 ng PCR per 20 μL of multiplexed samples. Paired-end read sequencing was performed on the Illumina MiSeq platform at the Functional Genomics Center, Zürich.

Analysis of microbiota composition. The raw sequencing data were processed using USEARCH (version 11.0.667)[40]. Paired reads were merged and filtered using the fastq_mergepairs command with default settings. Merged reads were filtered using the fastq_filter command (−fastq_maxee 0.1) and only merged reads with perfect primer matches and a minimum length of 100 bp were selected. Sequences were de-replicated using the fastx_uniques command and clustered into zero-nucleotide sequences using the fastx_truncate command, which also removed chimeric sequences[46]. All OTU abundances for each sample were quantified using the otutab command (−strand both; −id 0.97). Taxonomic annotation was performed by querying zOTU sequences against the SILVA database (version 128) using the usearch_global command (−id 0.90; −maxaccepts 20; −mtransform 0.5; −strand both; −maxns 2; −id 0.97). The PhyloWorkbench (Cytoscape, Jena, Germany) was used for interactive exploration and visualization of species abundance and its potential contributions to 16S rRNA gene expression.

Data analysis. RNA-Seq read quality were checked with FastQC (Babraham Bioinformatics), which computes various quality metrics for raw reads. Reads were aligned to the genome and transcriptome with TopHat v 1.3.3 (https://ccb.jhu.edu/software/tophat/manual.shtml). Before mapping, the low-quality ends of the reads were clipped (3 bases from the read start and 10 bases from the read end). TopHat was run with default options. The fragment length parameter was set to 100 bases, with an SD of 100 bases. Based on these alignments, the distribution of the reads across genomic features was analyzed. For seed enrichment analysis, only genes with FPKM above 1.0 were considered as targets. For cumulative distribution function calculations, log2 fold-change values were corrected for 3′ UTR length biases.

Histopathological examination and immunofluorescence. Small intestines were fixed in 3% paraformaldehyde and embedded in paraffin. Sections were cut from paraffin-embedded tissues at a 3.5 μm thickness, deparaffinized, and antigen retrieved by boiling them in Citrate buffer (pH 6). The sections were permeabilized and blocked in PBS solution with 0.1% Triton X-100, 1% BSA, and 5% donkey or goat serum depending on the antigen host. Sections were stained with primary antibodies overnight at 4°C, followed by secondary antibody incubation at room temperature for 1 h.

The lysozyme-positive cells in the crypt were quantified and normalized to DAPI-positive cells in crypt (60 crypt for each mouse) using NIH ImageJ software (http://rsweb.nih.gov/ij/download/) and normalized to crypt number. For intestinal histology analysis, sections were cut at 3.5 μm. A total of 5–6 sections, at least 400 μm apart, were cut from each mouse and further processed with routine H&E. All small intestinal sections were scanned using a ×20 objective of the Pannoramic 250 Slide scanner (3D Histech) or by Confocal microscopy. Villous height and crypt depth were measured in at least 30 well-oriented, villus-crypt units per mouse using ImageJ and CaseViewer.

Alcin Blue staining. For Alcin Blue staining, the intestinal tissues were fixed in Carnoy’s solution (60% ethanol, 30% chloroform, and 10% glacial acetic acid) for 4 h at room temperature. Subsequently, the tissues were transferred in 100% ethanol overnight and further processed for paraffin sectioning. Following deparaffinization, slides were stained in Alcin blue solution (3%, pH 2.5, Sigma) for 30 min. Slides were washed in tap water for 2 min, counterstained with nuclear fast red solution (Sigma-Aldrich) for 5 min, and followed by dehydration in ethanol gradient solution. Goatel cells were quantified using the Cell counter ImageJ software and normalized to crypt numbers.

FITC intestinal permeability. Female mice received FITC by oral gavage at a concentration of 60 mg/kg body weight after 6 h of water fasting. After 4 h the blood was collected and centrifuged for plasma separation. The plasma was diluted 1:1 in PBS and FITC concentration was determined by Tecan Fluorescence with an excitation of 485 nm and emission wavelength of 528 nm.

ROS assay. For ROS measurement the kit from MyBioSource (MBS723353) was used. Briefly, proximal jejunum was washed in ice cold PBS and sonicated on ice in the buffer provided. The samples were subsequently centrifuged and assayed immediately following the manufacturer’s protocol. Data were normalized to protein concentration.

INOS Elisa. For INOS measurement in the jejunal intestine the kit from Lubioscience (STA-347) was used. Briefly, tissues were resuspended in 1 mL PBS and sonicated on ice. The samples were subsequently centrifuged at 10,000 × g for 5 min and assayed immediately following the manufacturer’s protocol.

Intestinal epithelial cell proliferation and turnover. For assessing intestinal epithelial cell proliferation, female mice were injected with Bromo-2′-deoxyuridine (BrDU) in PBS intraperitoneally (100 μg/g body weight) and euthanized 1.5 h later. BrDU-labeled cells in the jejenum were detected using BrDU antibody. The BrDU-
positive cells in the crypt were quantified and normalized to crypt number (60 crypt per mouse) using NIH Image software. For analyzing epithelial cell turnover, BrdU injection at the same dose was given at the same day. After 36 h, mice were sacrificed and BrdU-positive cells were detected using BrdU antibody.

**EDU flow cytometry.** Female mice were injected with 20 mg/kg of body weight EDU in PBS. After 1 h incubation mice were euthanized and crypts were isolated as previously described. Crypts were dissociated in single cells using TRIPLEX enzyme for 2 min at 37 °C. After, cells were fixed and incubated with anti-EDU antibody following the kit instruction protocol of Click-iT® EDU Flow Cytometry Assay Kits. Cells were counterstained with Hoechst and EDU/positive single cells were measured using Flow cytometry BD LSRFortessa.

**TUNEL assay.** For Tunel assay a commercially available kit Click-iT® TUNEL Alexa Fluor® Imaging Assay was used with some modifications. For this, freshly cut paraffin blocks were deparaffinized and rehydrated in alcohol gradients and fixed in paraﬁn in 15 min at RT. Subsequently, slides were digested in ProteinaseK for 15–20 min and fixed again for 5 min in 4% PFA. After this step, the normal kit instruction protocol was followed and slides were counterstained with Hoechst for the nuclei.

**Mouse small intestine organoid culture and virus infection.** Small intestinal organoids were established from isolated crypts of the proximal intestine from 15–18 week old mice by mechanical dissociation and maintained in Corning recovery solution and mechanically dissociated in Basal Media (Advanced DMEM/F12 supplemented with Pen/streptomycin, 10 mmol/L HEPES, Glutamax) supplemented with N-acetylcysteine, 1 × B27, 1 × N2, 50 ng/ml EGF, 1 μg/ml R-spondin, and 0.1 μg/ml Noggin. All growth factors were purchased from Peprotech. For virus infection experiments, organoids were harvested in Corning recovery solution and mechanically dissociated in Basal Media using a narrowed Pasteur Pipette for 5–7 times up and down. After, organoids were centrifuged at 250 × g and resuspended in 400 μl full growth medium containing 100 × 10^5 MOI virus for 100 μl matrigel. The mixture was incubated at 37 °C for 5 h, with continuous mixing every 30 min. Next, the mixture was centrifuged at 250 × g for 5 min and organoids were seeded in matrigel and cultured for 3 days before harvesting for RNA isolation.

**Cell fractionation.** Crypts were isolated and the cell pellet was resuspended in Hypotonic Lysis Buffer (HLB, 10 mM HEPES, 1.5 mM MgCl₂, 10 mM KC1, 1 mM DTT, and fresh protease inhibitor) and incubated on ice for 30 min. 6 μl 10% NP40 per 100 μl HLB (100 μl of HLB for 1 M cells) was added after the incubation and pipetting up and down. An aliquot was taken as input and then spun down at 13 K rpm 35 s at 4 °C. The supernatant was saved as a cytosolic extract. Optionally, the nuclei were then washed in HLB to remove any contaminating cytoplasm and re-sedimented. The nuclei were then lysed in 20 μl Lysic Lysis Buffer (NLB, 10 mM HEPES, 100 mM KC1, 3 mM MgCl₂, 0.1 mM EDTA, 1 mM DTT, and fresh protease inhibitor) and incubated on ice for 30 min. Extra 1/10th volume of 4 M (NH₄)₂SO₄ was added over 30 min. After the lysate was spun at 13 K rpm 15 min at 4 °C, the supernatant was saved as the nuclear extract. The nuclear fraction, cytoplasmic fraction and input, were fractioned and loaded in Laemmli buffer in 12% SDS gel, and membranes were incubated in H3 (CST 4499), and β-CATENIN (CST 8814) antibodies overnight at 4 °C.

**TopFLASH assay.** The TOPFLASH assay was performed in 96-well plates. For each well, cells were transfected with 0.15 μg TOPFLASH (or FOPFLASH with mutant TCF binding sites) and 0.02 μg PRL-SV40P using Lipofectamine 2000. For co-transduction with other plasmids, 6 h before transfection cells were transduced with 50 MOI Ad-miR-802 or Ad-Tmed9. For Tmed9 knockdown experiments 20 nm si-Tmed9 were transfected with RNAmax 6 h before transfection. The luciferase activity was measured 24 h later using the Dual-Glo Luciferase Assay System (E2940; Promega) according to the manufacturer’s protocol. The TOPFLASH or FOPFLASH activity was normalized to Renilla luciferase signals. For transduction experiments, data were normalized to the Ad-ctrl treated samples with the equivalent MOI. For siRNA transfection experiments, data were normalized to the si-Ctrl treated samples with the same concentration (20 nm).

**Salmonella Typhimurium colonization assay.** For the mono-colonization experiments, females, age-matched, littermate controlled, specify pathogen-free mice were orally gavaged with 10⁶ CFU of Salmonella Typhimurium strain SL1344.38 The bacterial culture was initially grown in LB medium containing 0.3 M NaCl for 12 h at 37 °C and then subcultured for 4 h in fresh medium at a 1:20 dilution. The bacteria were spun down and resuspended in an equal volume of PBS prior to infection. Mice were sacrificed after 48 h and intestinal tissues were snap-frozen for RNA extraction. Small intestinal colonization levels were measured by dilution plating of luminal contents. Bacterial levels in spleen, feces, and mLN were determined by dilution plating of homogenized tissue.

**Data availability**
Sequencing data have been deposited in the ArrayExpress under accession code: GSE148198, https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM4456008. Microbiome sequencing data have been deposited in European Nucleotide Archive (ENA) database under accession code: PRJEB43950. The authors declare that the data supporting the findings of this study are available within the paper and its supplementary information files. All remaining data will be available from the corresponding author upon reasonable request. Source data are provided with this paper.

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### References
1. Bartel, D. P. MicroRNAs: target recognition and regulatory functions. Cell 148, 1127–1137 (2012).
2. Mendell, J. T. & Olson, E. N. MicroRNAs in stress signaling and human disease. Cell 148, 1172–1187 (2012).
3. Gerlach, C. V. & Vaidya, V. S. MicroRNAs in injury and repair. Arch. Toxicol. 91, 2781–2797 (2017).
4. Cullen, B. R. MicroRNAs as mediators of viral evasion of the immune system. Nat. Immunol. 14, 205–210 (2013).
5. Kornfeld, J. W. et al. Obesity-induced overexpression of miR-802 impairs glucose metabolism through silencing of Hnf1b. Nature 494, 111–115 (2013).
6. Zhang, X. Y., Mu, J. H., Liu, L. Y. & Zhang, H. Z. Upregulation of miR-802 suppresses gastric cancer oncogenicity via targeting RAB23 expression. Eur. Rev. Med. Pharmacol. Sci. 21, 4071–4078 (2017).
7. Zhang, G. et al. Obesity-induced overexpression of miR-802 impairs insulin transcription and secretion. Nat. Commun. 11, 1822 (2020).
8. Yuan, F. & Wang, W. MicroRNA-802 suppresses breast cancer proliferation through downregulation of FoxM1. Mol. Med. Rep. 12, 4647–4651 (2015).
9. Hoo, P. & Waxman, D. J. Functional roles of sex-biased, growth hormone-regulated microRNAs miR-1948 and miR-802 in young adult mouse liver. Endocrinology 159, 1377–1392 (2018).
10. Wolenski, F. S. et al. Identification of microRNA biomarker candidates in urine and plasma from rats with kidney or liver damage. J. Appl. Toxicol. 37, 278–286 (2017).
11. Kapoor, B. B., Smit, H. & Verighina, I. A. The alimentary canal and digestion in teleosts. Adv. Mar. Biol. 48, 109–239 (1975).
12. Gehart, H. & Clevers, H. Tales from the crypt: new insights into intestinal stem cells. Nat. Rev. Gastroenterol. Hepatol. 16, 19–34 (2019).
13. Clevers, H. C. & Bevins, C. L. Paneth cells: maestros of the small intestinal crypts. Annu. Rev. Physiol. 75, 289–311 (2013).
14. Muller, T. D. et al. Glucagon-like peptide 1 (GLP-1). Mol. Metab. 30, 72–130 (2019).
15. Wilson, C. L. et al. Regulation of intestinal alpha-defensin activation by the metaalloproteinase matrilysin in innate host defense. Science 286, 113–117 (1999).
16. Vaishnav, S. et al. The antibacterial lectin RegIII gamma promotes the spatial segregation of microbiota and host in the intestine. Science 334, 255–258 (2011).
17. Mukherjee, S. & Hooper, L. V. Antimicrobial defense of the intestine. Immunity 42, 28–39 (2015).
18. Salzman, N. H., Ghosh, D., Huttner, K. M., Paterson, Y. & Bevins, C. L. Protection against enteric salmonellosis in transgenic mice expressing a human intestinal defense. Nature 422, 522–526 (2003).
19. Dan, L. et al. Expression of recombinant human lysozyme in bacterial artificial chromosome transgenic mice promotes the growth of Bifidobacteria and inhibits the growth of Salmonella in the intestine. J. Biotechnol. 272-273, 33–39 (2018).
20. Adolph, T. E. et al. Paneth cells as a site of origin for intestinal inflammation. Nature 503, 272–276 (2013).
21. Al-Barazie, R. M. et al. Cholinergeic activation enhances resistance to oral Salmonella infection by modulating innate immune defense mechanisms at the intestinal barrier. Front. Immunol. 9, 551 (2018).
22. Saillaja, B. S. et al. The regulatory niche of intestinal stem cells. J. Physiol. 594, 4827–4836 (2016).
26. Delgado, M. E., Grabinger, T. & Brunner, T. Cell death at the intestinal epithelial front line. FEBs J. 283, 2701–2719 (2016).
27. Bensalem, M., Vandeputte, C. & Laybutt, D. R. Mechanisms of β cell dedifferentiation in diabetes: recent findings and future research directions. J. Endocrinol. 236, R109–R143 (2018).
28. Kim, C. K. et al. Intestinal stem cell resuscitation by enterocyte precursors. Stem Cell Investig. 3, 49 (2016).
29. Shinar, J. et al. The intestinal epithelial cell differentiation marker intestinal alkaline phosphatase (ALP) is selectively induced by histone deacetylase inhibitors (HDACi) in colon cancer cells in a Kruppel-like factor 5 (KLF5)-dependent manner. J. Biol. Chem. 290, 15392 (2015).
30. Muncan, V. et al. Blimp1 regulates the transition of neonatal to adult intestinal epithelium. Nat. Commun. 2, 452 (2011).
31. Harper, J., Armes, A., Andrews, R. M., Bikoff, E. K. & Robertson, E. J. The transcriptional repressor Blimp1/Prdm1 regulates postnatal reprogramming of intestinal enterocytes. Proc. Natl Acad Sci. USA 110, 10585–10590 (2011).
32. Gaudioin, S. J. et al. IL-22 receptor signaling in Paneth cells is critical for their maturation, microbiota colonization, Th17-related immune responses, and the Salmonella immunity. Micro. Immunol. Inpress. https://doi.org/10.1038/s41385-020-03084-5 (2020).
33. Gazit, R. et al. Transcriptome analysis identifies regulators of hematopoietic stem and progenitor cells. Stem Cell Rep. 1, 266–280 (2013).
34. Haus, G. et al. The transcription factor FoxM1 activates Nurrl to promote intestinal regeneration after ischemia/reperfusion injury. Exp. Mol. Med. 51, 132 (2019).
35. Park, J. S. et al. Requirement of ATR for maintenance of intestinal stem cells in aging Drosophila. Ageing 7, 307–318 (2015).
36. Saenz-Robles, M. T. et al. Intestinal hyperplasia induced by simian virus 40 large tumor antigen requires E2F2. J. Virol. 81, 13191–13199 (2007).
37. Broman, L. et al. A comparison of proliferation markers (Ki67, Ki-67, PCNA) determined at each cell position in the crypts of normal human colon mucosa. Eur. J. Histoch. 40, 89–100 (1996).
38. Batts, L. E., Polk, D. B., Dubois, R. N. & Kulesza, H. Bmp signaling is required for intestinal growth and morphogenesis. Dev. Dyn. 235, 1563–1570 (2006).
39. Willert, K. et al. Wnt proteins are lipid-modifying regulators of intestinal organoid model. J. Cell Biol. 219, 1918–1927 (2012).
40. Stamos, J. L. & Weiss, W. I. The β-catenin destruction complex. Cold Spring Harb. Perspect. Biol. 5, a007898 (2013).
41. Kishida, S. et al. Axiin, a negative regulator of the wnt signaling pathway, directly interacts with adenomatous polyposis coli and regulates the stabilization of beta-catenin. J. Biol. Chem. 273, 10823–10826 (1998).
42. Zwarycz, B. et al. IL-22 inhibits epithelial stem cell expansion in an ileal Organoid model. Cell. Mol. Gastroenterol. Hepatol. 7, 1–17 (2018).
43. Cohen, S. M., Brennan, J. & Stark, A. Denoising feedback loops by thresholding—a new role for microRNAs. Genes Dev. 20, 2769–2772 (2006).
44. Caliceti, C. et al. ROS, Notch, and Wnt signaling pathways: crosstalk between three major regulators of cardiovascular biology. Biomed. Res. Int. 2014, 8 (2014).
45. Maiti, S. et al. Effective control of Salmonella infections by employing combinations of recombinant antimicrobial human β-Defensins hBD-1 and hBD-2. Antimicrob. Agents Chemother. 58, 6996–6993 (2014).
46. Looen, L. M. P. et al. REG8-dependent mice have altered mucus distribution and increased mucosal inflammatory responses to the microbiota and enteric pathogens in the ileum. Mucosal Immunol. 7, 939–947 (2014).
47. Hooper, L. V., Stappenbeck, T. S., Hong, C. V. & Gordon, J. I. Angiogenins: a new class of microbial proteins involved in innate immunity. Nat. Immunol. 4, 269–273 (2003).
48. Rawls, J. F., Mahowald, M. A., Ley, R. E. & Gordon, J. I. Reciprocal gut microbiota transplants from zebraﬁsh and mice to germ-free recipients reveal host habitat selection. Cell 127, 423–432 (2006).
49. Salzman, N. H. et al. Enteric defensins are essential regulators of intestinal microbiology. Nat. Immunol. 11, 76–83 (2010).
50. Chuang, K., Nguyen, E., Sergeev, Y. & Badea, T. C. Novel heterotypic Rox sites and increased mucosal releasing factors reveal effective function of β-defensin. Science 332, 659–657 (2010).
51. Araki, K., Araki, M. & Yamamura, K. Site-directed integration of the cre gene mediated by Cre recombinase using a combination of mutant lox sites. Nucleic Acids Res. 30, e100 (2002).
52. Röder, P. V. et al. The role of SGLT1 and GLUT2 in intestinal glucose transport and sensing. PLoS ONE 9, e89977 (2014).
53. Frazer, D. M. et al. Ferroportin is essential for iron absorption during suckling, growth and increased mucosal invasive responses to the microbiota and enteric infections by employing combinations of recombinant antimicrobial human β-Defensins hBD-1 and hBD-2. Antimicrob. Agents Chemother. 58, 6996–6993 (2014).
54. Altay, G. et al. Self-organized intestinal epithelial monolayers in cryopreservation and vilius-like domains show effective barrier function. Sci. Rep. 9, 10140 (2019).
55. Neffor, C. M. et al. A versatile strategy for isolating a highly enriched population of intestinal stem cells. Stem Cell Rep. 6, 321–329 (2016).
56. von Furstenberg, R. J. et al. Sorting mouse jejunal epithelial cells with CD24 and CD320 identifies a population with characteristics of intestinal stem cells. Am. J. Physiol. Gastrointest. Liver Physiol. 299, G2769–G2772 (2006).
57. Apprill, A. Mc, Nally, S., Parsons, R. & Weber, L. Minor revision to V4 region SSU rRNA gene primer greatly increases detection of SAR11 bacterioplankton. Aquat. Microb. Ecol. 75, 129–137 (2015).
58. Parada, A. E., Needham, D. M. & Fuhrman, J. A. Every base matters: assessing small subunit rRNA primers for marine microbiomes with high-throughput communities, time series and global field samples. Environ. Microbiol. 18, 1403–1414 (2016).
85. Edgar, R. C. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **26**, 2460–2461 (2010).
86. Edgar, R. C. UNOISE2: improved error-correction for Illumina 16S and ITS amplicon sequencing. Preprint at bioRxiv https://doi.org/10.1101/081257.
87. Sato, T. et al. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature* **459**, 262–265 (2009).
88. Hoiseth, S. K. & Stocker, B. A. D. Aromatic-dependent *Salmonella typhimurium* are non-virulent and effective as live vaccines. *Nature* **291**, 238–239 (1981).

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**Author contributions**

A.G., B.Y., and K.H. contributed equally to the project. A.G., B.Y., K.H., M.S. designed the study. A.G., B.Y., K.H., R.D., S.G., and P.S. performed the experiments and analyzed data. M.Z. and S.S. performed and analyzed the microbiota studies. M.F., B.Y., A.G., and W.-D.H. carried out and analyzed the *Salmonella* infection experiments. A.G. and B.Y. performed the bioinformatic and statistical analysis. A.G. and M.S. wrote the manuscript. All authors read and provided input on the manuscript.

**Competing interests**

The authors declare no competing interests.

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**Additional information**

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