Supplementary Methods

RT-qPCR for miRNAs

For RT-qPCR miRNA analysis, reverse transcription was performed using miRCURY LNA RT Kit (339340) and quantitative polymerase chain reaction (qPCR) for hsa-let-7b-5p (miRCURY LNA primer set, YP00204750), hsa-miR-16-5p (YP00205702), hsa-miR-22-3p (YP00204606), hsa-miR-23a-3p (YP00204772), hsa-miR-26b-5p (YP00204772), hsa-miR-150-5p (YP00204772), hsa-miR-4454 (YP02114119), hsa-miR-451a (YP02119305), cel-miR-39-3p (YP00203952), RNU1A1 (YP00203909), RNU5G (YP00204772) and 5S rRNA (YP00203906) using miRCURY LNA SYBR Green PCR Kit (339346, Qiagen, Hilden, Germany) on CFX384 real-time PCR detection system (Bio-Rad, Hercules, California, USA). The qPCR conditions applied were 95°C for 10 min, and 40 cycles of 95°C for 10 s and 60°C for 1 min, followed by melting curve analysis. qPCR reactions were performed in quadruplicates and cel-miR-39-3p (spike-in), RNU5G, RNUA1 and 5S rRNA were used as reference genes.

Cell culture and transfection

Human NCM356 colonic epithelial cell line (Incell, San Antonio, Texas, USA) and peripheral blood mononuclear cells (PBMC) were cultured in M3Base medium (M300F) and Roswell Park Memorial Institute medium 1460 (RPMI-1460), respectively, supplemented with 10% fetal bovine serum (FBS) (10270-106; Life Technologies, Carlsbad, California, USA) and 1% Penicillin-Streptomycin (15070-063) in a humidified incubator at 37°C with 5% CO_2. Treatment with cytokines was performed at the concentration of 50 ng/ml using recombinant human IL-12 p70 (Peprotech, 200-12H), IL-18/IL-1F4 (R&D, 9124-IL-010), FGF-21 (R&D, 2539-FG-025) or 4-1BB Ligand/TNFSF9 (R&D, 2295-4L-025). For transfections, cells were plated in 6-well tissue culture plates and transfected with hsa-miR-20a-5p (YM00472205), hsa-miR-23a-3p (YM00470983), hsa-miR-26b-5p (YM00472485), hsa-miR-28-5p (YM00471553), hsa-miR-

130a-3p (YM00472237), hsa-miR-150-5p (YM00470312) miRCURY LNA miRNA mimics, miRNA control (YM00479902, Qiagen) at a final concentration of 20nM or with siRNA against Drosha (Dharmacon, ON-TARGETplus Human DROSHA siRNA, SMARTPool, L-016996-00-0005) or the respective siControl (Dharmacon, ON-TARGETplus non-targeting control pool, D-001810-10-05) using Lipofectamine RNAiMAX (13778-150; Invitrogen, Carlsbad, CA, USA) according to manufacturer's instructions. PBMCs were treated with miRCURY LNA microRNA inhibitors (20nM) for hsa-miR-150-5p (YI04101205), hsa-miR-26b-5p (YI04104759) or negative inhibitor control (YI00199007, Qiagen) without transfection reagent. Cells were collected 24 h later for RNA extraction.

RNA extraction, RT-qPCR for genes

Tissues were homogenized in Trizol (15596026, Invitrogen) and total RNA was isolated according to established protocols. Total RNA from cell cultures was isolated using QIAshredder (79656) and RNeasy Plus Mini Kit (74136, Qiagen) according to manufacturer's instructions. Reverse transcription was performed using iScript Reverse Transcription Supermix (170-8841). cDNAs were subjected to quantitative polymerase chain reaction (qPCR) using iTaq Universal SYBR Green Supermix (172-5124, Bio-Rad) on a CFX384 real-time PCR detection system (Bio-Rad) and the primers listed in Supplementary Table 1. The qPCR conditions were 95°C for 30 s, and 45 cycles of 95°C for 15 s, 55°C for 10 s and 72°C for 30 s, followed melting curve analysis. β-actin and GADPH were used as reference genes. Results are derived from 3 independent experiments performed in quadruplicates.

Immunoblot analysis

Tissues and colonoids were homogenized using RIPA cell lysis buffer (9806, Cell Signaling Technology) supplemented with protease inhibitors (04693132001, Roche). Western blots of electrophoresed (SDS-PAGE) cell lysates were probed with Ago2 (2897), Drosha (3364, Cell

Signaling Technology), Dicer (sc-136981, Santa Cruz) and alpha-Tubulin (T5168, Sigma) monoclonal antibodies, following standard procedures.

Construction of 3'UTR reporter vectors

miRNA target predictions were performed using the TargetScan 7.2 software [3]. All 64 miRNAs found to be induced by FMT were screened against the 3'UTR of FGF21, IL-12B, IL-18 and TNFRSF9. The 3'UTR sequences of FGF21, IL-12B, IL-18 and TNFRSF9 were amplified using Q5 High-Fidelity DNA Polymerase (M0491; NEB, Ipswich, Massachusetts, USA) according to the manufacturer's instructions, using the primers listed in Supplementary Table 2. The amplified 3'UTR sequences were cloned into psi-CHECK2 vector (C8021; Promega, Madison, Wisconsin, USA), downstream of the *Renilla Luciferase* gene. *Firefly Luciferase* gene expressed by this vector independently of the 3'UTR sequences was used as reference. The 3'UTRs at the corresponding miRNA target sequence (miR-26b for FGF21; miR-23a for IL-12B; miR-150 for IL-18; miR-28 for TNFRSF9) were mutated (deletion mutants) using the QuikChange II site-directed mutagenesis kit (200524; Aligent Technologies, Santa Clara, California, USA) according to the manufacturer's instructions. Primers used for mutagenesis are listed in Supplementary Table 3. Cloned sequences and mutations were verified by DNA sequencing.

3'UTR reporter assays

NCM356 cells were plated in a 6-well plate and transfected with the 3'UTR reporter vectors using Lipofectamine 3000 (L3000-008; Invitrogen, Carlsbad, California, USA). Cells were transfected, 24 h later, with the respective microRNA mimic or the control (Qiagen, Hilden, Germany) by using Lipofectamine RNAiMAX (13778-150). 20,000 cells/well were plated in Nunclon Delta Surface 96-well plate (136101; Thermo Scientific, Waltham, Massachusetts, USA) and Luciferase activity (luminescence) was measured using the Dual-Luciferase

Reporter Assay kit (E1980; Promega, Madison, Wisconsin, USA), on a Cytation 3 multi-mode reader (BioTek, Winooski, Vermont, USA), according to the manufacturer's instructions. *Renilla* Luciferase activity was normalized against *Firefly* Luciferase activity per well. Results are derived from 3 independent experiments performed in quadruplicates.

Luminescence cell viability assay

Cells were transfected with the respective miRNAs and plated in quadruplicates in 96-well plate ($25x10^3$ cells/well). 48h following transfection, cells were treated with TcdA and/or TcdB (at the concentrations of 10 or 50 pM). In another set of experiments, 48h following transfection, the cells were treated with the respective cytokines (at at the concentration of 50ng/ml) and 30 min later TcdA and/or TcdB was added (at at the concentrations of 10 or 50 pM). Following treatment, growth was assessed at 48h using the CellTiter Glo Luminescence Cell Viability Assay (G7573, Promega) according to the manufacturer's instructions. Luminescence readings were acquired with CLARIOstar plate reader (BMG Labtech). Data were expressed as mean luminescence (arbitrary units) ± s.e.m. (control cells were set as 100%).

IncuCyte Live-Cell growth assay

Cells were transfected with the respective miRNAs and plated in quadruplicates in 96-well plate ($25x10^3$ cells/well). 48h following transfection, cells were treated with TcdA and/or TcdB (at the concentrations of 10 or 50 pM). In another set of experiments, 48h following transfection, the cells were treated with the respective cytokines (50ng/ml) and 30 min later TcdA and/or TcdB was added (10 or 50 pM). Following treatment, growth was assessed using the IncuCyte Live-Cell analysis system, where images with 10X magnification were captured every 2 hours for total of 3 days and analysed. Data were expressed as mean % confluency ± s.e.m.

IncuCyte Live-Cell apoptosis assay

Cells were transfected with the respective miRNAs and plated in quadruplicates in 96-well plate ($25x10^3$ cells/well). 48h following transfection, cells were treated with TcdB (10 or 50 pM) and Incucyte Caspase-3/7 Red Dye for Apoptosis (Essen Bioscience, 4704) was added in the cells in a final concentration of 0.5 µM. Apoptosis was assessed using the IncuCyte Live-Cell analysis system, where images with 10X magnification were captured every 2 hours for total of 2 days and analysed. Data were expressed as mean % confluency ± s.e.m.

ApoTox-Glo Assay

Cells were transfected with the respective miRNAs and plated in quadruplicates in 96-well plate ($25x10^3$ cells/well). 48h following transfection, cells were treated with TcdB (10 or 50 pM). Cytotoxicity and cell survival was assessed at 2, 24 and 48h using the ApoTox-Glo Triplex Assay (Promega, G6320). Briefly, 20µl of Viability/Cytotoxicity Reagent containing both GF-AFC Substrate and bis-AAF-R110 Substrate, previously warmed at 37°C, was added per well. Contents were mixed briefly by orbital shaking at 300–500rpm for 30 seconds and incubated for 30 minutes at 37°C. Fluorescence was measured at 400Ex/505Em for cell viability and 485Ex/520Em for cell cytotoxicity, using the CLARIOstar plate reader (BMG Labtech).

Phalloidin actin staining

Cells were transfected with LNA miRNA mimics (20nM) for miR-23a-3p and miR-150-5p or cel-miR-39 as the control and plated in 8-well tissue culture coverslips (ibidi, 80826), pre-coated with 50 µg/ml collagen I (Life Technologies, A10483-01). 48h after transfection, cells were treated with a combination of toxins A and B (10pM) for 4h. Following treatment, cells were fixed in 4% formaldehyde for 15 min at room temperature and rinsed (3x5min) with PBS. Permeabilization was achieved with 0.5% Triton X-100 in PBS, for 10 min at room temperature

followed by 100mM glycine (in PBS, 0.1% Tween-20) for 2 min at room temperature and rinsed with PBS. Cells were incubated in Image-IT FX Signal Enhancer Ready Probes Reagent (Life Technologies, R37107) for 20 min at room temperature and then rinsed with 0.1% Triton X-100 in PBS, 20 min in blocking solution (PBS, 0.1%Triton X-100, 2% BSA) and rinsed with 0.1% Triton X-100 in PBS. Actin staining was performed with AlexaFluor 488 phalloidin (Life Technologies, R37110) and nuclei staining with NucBlue live ReadyProbes reagent (Life Technologies, R37605), in blocking solution, for 30 min at room temperature protected from light. Cells were rinsed once with 0.1% Triton X-100 in PBS and washed (3x5min) with PBS. Images were captured with a fully automated Zeiss AxioObserver Z1 inverted microscope equipped with AxioCam MRm high-resolution camera, using the AxioVision SE64 image acquisition software.

Supplementary Table 1. qPCR primers.

Gene	Forward	Tm (°C)	Reverse	Tm (°C)	Size (bp)
FGF21	5'-GGGAGTCAAGACATCCAGGT-3'	59	5'-TGTATCCGTCCTCAAGAAGCA-3'	60.8	116
IL18	5'-AGGAAATCGGCCTCTATTTG-3'	59.7	5'-CCATACCTCTAGGCTGGCTATC-3'	59.3	110
IL12B	5'-CATTGAGGTCATGGTGGATG-3'	59.8	5'-GGTGGGTCAGGTTTGATGAT-3'	59.6	93
AGO2	5'-GACACGAAAATCACCCACCC-3'	59.1	5'-GGACGTGATAGTGCGAAGG-3'	58	94
DICER1	5'-CTGCAAATGTACCCCGTTCC-3'	59.2	5'-GTGACTCTGACCTTCCCGTC-3'	59.8	106
DROSHA	5'-AGGACAGAAGGAAAAGAGCCA-3'	58.9	5'-ACAGTGTAGGTTCGGGCATG-3'	60	83
TNFRSF9	5'-CTGCCGATTTCCAGAAGAAG-3'	59.5	5'-GAAAGCTGTGATAGCGGATGA-3'	60.4	120
pri-miR-16-5p	5'-GCACGTAAATATTGGCGTTAAG-3'	56.5	5'-CACAACTGTAGAGTATGGTCAACCT-3'	61.3	88
pri-miR-23a-3p	5'-ATCACATTGCCAGGGATTTC-3'	55.2	5'-AGCTAAGCCCTGCTCCTCAG-3'	61.4	118
Actin	5'-CCCAGCACAATGAAGATCAA-3'	59.6	5'-ACATCTGCTGGAAGGTGGAC-3'	60.1	103
GAPDH	5'-ATGTTCGTCATGGGTGTGAA-3'	59.8	5'-GGTGCTAAGCAGTTGGTGGT-3'	60.2	89
pri-miR-150-5p	5'- GGGCTCAGACCCTGGTACA-3'	61	5'- GAGTACAGGGAGGGGAGGTC-3'	63.4	109
mAgo2	5'-CCACCCCACTGAGTTTGACT-3'	59.5	5'-TTGTCATCCCAAAGCACGTG-3'	59.1	93
mDicer1	5'-TCTGCAGGCTTTTACACACG-3'	58.8	5'-ACAGCCACAGTGTAGGTTCT-3'	58.6	88
mDrosha	5'-GGACAGAAGGGAAAGAGCCT-3'	59	5'-CCAAAATCGCATCTCCCAGG-3'	59	89
mActin	5'-CCAACCGTGAAAAGATGACC-3'	60.4	5'-CCATCACAATGCCTGTGGTA-3'	60.4	120
mGAPDH	5'-GTGTTTCCTCGTCCCGTAGA-3'	60.1	5'-AATCTCCACTTTGCCACTGC-3'	60.3	108

Supplementary Table 2. 3'UTR cloning primers.

Gene	Forward	Tm (°C)	Reverse	Tm (°C)
FGF21	5'-CT**CTCGAG** GCCAGAGGCTGTTTACTATGA-3'	57.1	5'-GC**GCGGCCGC** TCCTCCTCTGGAACTCTTTATTATC-3'	58.4
IL18	5'-CT**CTCGAG** GAGGATGAATTGGGGGATAGA-3'	59.8	5'-GC**GCGGCCGC** AAACATAAAAATTCAGACAGTTCACA-3'	58.7
IL12B	5'-CT**CTCGAG** TGATCCAGGATGAAAATTTGG-3'	60.1	5'-GC**GCGGCCGC** GAAGAGTTTTTATTAGTTCAGCCTCA-3'	58.3
TNFRSF9	5'-CT**CTCGAG** CTGCCGATTTCCAGAAGAAG-3'	68	5'-GC**GCGGCCGC** GAGGGTAGCAAGGATGTGGA-3'	76

Supplementary Table 3. Mutagenesis Primers.

Gene	Sense	Tm (°C)	Antisense	Tm (°C)
FGF21	5'-TCTGGAACTCTTTATTATCT CGTAAGAAAAATAAAAAAATAAAT -3'	62.9	5'-ATTTATTTTTTTATTTTTC TTACGAGATAATAAAGAGTTCCAGA-3'	62.9
IL18	5'-GTAATCCCAGCCCTTGG CTGAGGCGG-3'	71.1	5'-CCGCCTCAGCCAAGGG CTGGGATTAC-3'	71.1
IL12B	5'-TGTCAGTACAAATAAAATTA AATTTGCATAATAGGGACTGATCC-3'	66.6	5'-GGATCAGTCCCTATTAT GCAAATTTAATTTTATTTGTACTGACA-3'	66.6
TNFRSF9	5'-GTTATAGTAGAACCAACTAA AAGCAGAAGTGACAGATACCCA-3'	78.7	5'-TGGGTATCTGTCACTTCT GCTTTTAGTTGGTTCTACTATAAC-3'	78.7

References

1. Polytarchou, C. et al. *Inflamm Bowel Dis* 2015; 21: 2533-9

2. Gu, Z. et al. *Bioinformatics* 2016; 32: 2847-9

3. Agarwal, V. et al. eLife 2015; 4: e05005