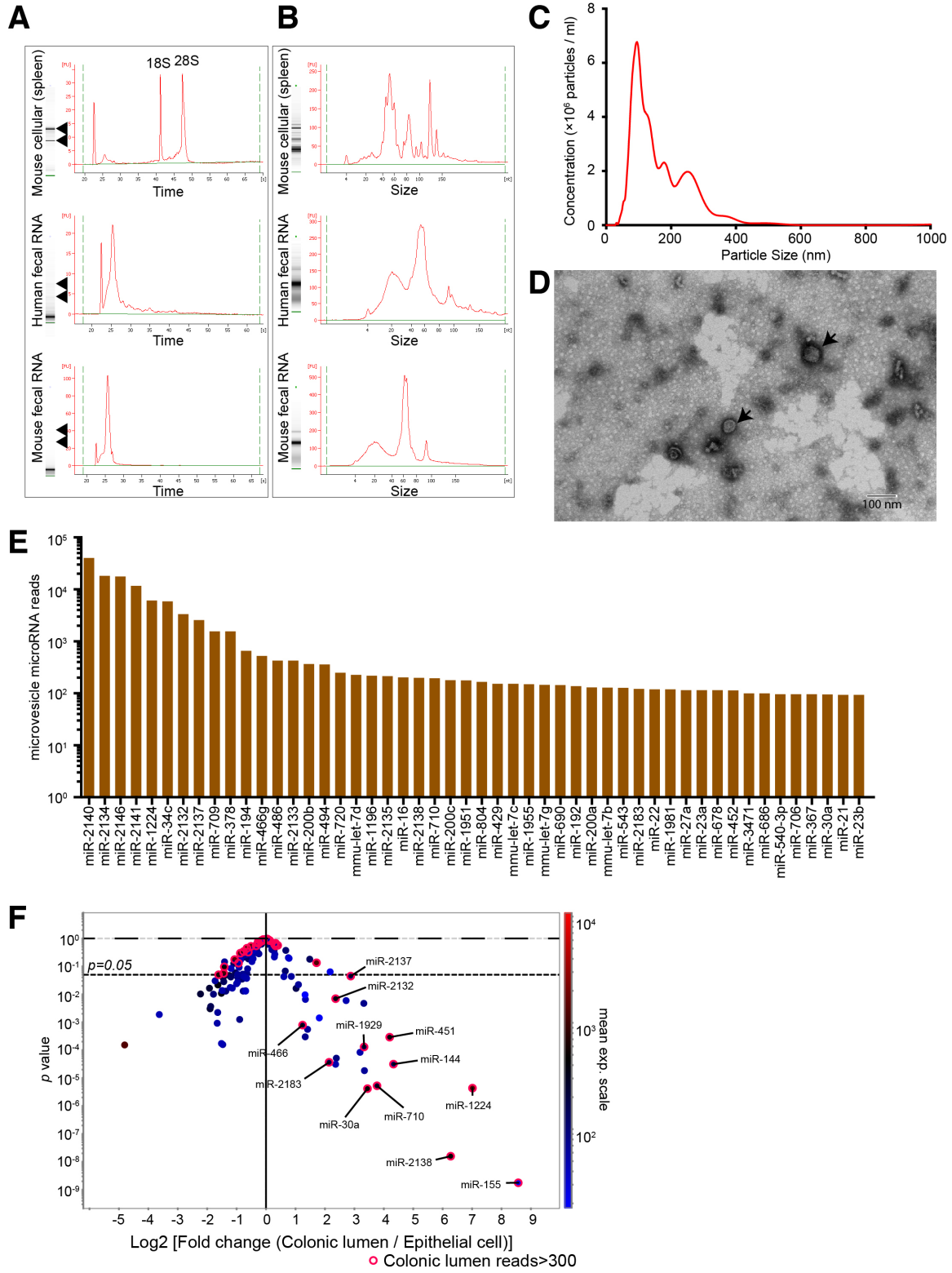# SUPPLEMENTAL FIGURES

Figure S1.

Figure S1. Identification of miRNA in the Feces

Related to Figure 1.

(A and B) RNA was isolated from human stool, mouse feces or mouse spleen cells. RNA size distribution was analyzed by: (A) Total RNA bioanalyzer and (B) Small RNA bioanalyzer.

(C and D) Germ-free SWGF mouse fecal suspensions were removed of debris and filtered. (C) Microparticle diameter distribution was measured by NanoSight in the 0.8 μm filtrates. (D) Exosome-sized (40-100 nm in diameter) extracellular vesicles were observed (arrow) by electron microscopy in the 0.2 μm filtrates. Representative of 6 samples from two experiments.

(E) RNA was isolated from the extracellular vesicles of germ-free SWGF feces and miRNA components in the RNA sample were measured by nanostring. The reads of the 50 most abundant miRNAs are shown.

(F) Volcano plots for miRNA levels detected in C57BL/6J mouse colonic luminal content (*n*=4) vs. colonic epithelial cells (*n*=4) based on nanostring; each dot represents one miRNA; *x*-axis: log 2 of fold change (colonic luminal content / epithelial cell); *y*-axis: *p* value comparing individual miRNAs between groups (unequal variance *t* test with Benjamini-Hochberg correction); the color of the dot indicates the mean expression level of the corresponding miRNA in both groups as shown in the side color scale bar. Red circles indicate miRNAs that are abundantly found in colonic lumen. Colonic abundant miRNAs that are significantly higher than epithelial cells are identified by number.

A

Dicer1^fl/fl

0.11%
0.03%
1.06%
2.31%
9.81%
17.19%
69.50%

- ▉ Bacteroidetes
- ▉ Firmicutes
- ▉ Proteobacteria
- ▉ Deferribacteres
- ▉ Tenericutes
- ▉ Actinobacteria
- ▉ Other

Dicer1^ΔIEC

0.33%
0.02%
2.13%
2.83%
14.13%
9.48%
71.09%

B

p=0.1887

UnWeighted UniFrac

Dicer1^fl/fl   Dicer1^ΔIEC

Prevotellaceae

C

p=0.0012

UnWeighted UniFrac

Dicer1^fl/fl   Dicer1^ΔIEC

Porphyromonadaceae

D

p=0.0005

UnWeighted UniFrac

Dicer1^fl/fl   Dicer1^ΔIEC

Lachnospiraceae

E

p value

p=0.05

OTU00145
OTU00190
OTU00150
OTU00192
OTU00045
OTU00025
OTU00015

cv of Dicer1^ΔIEC

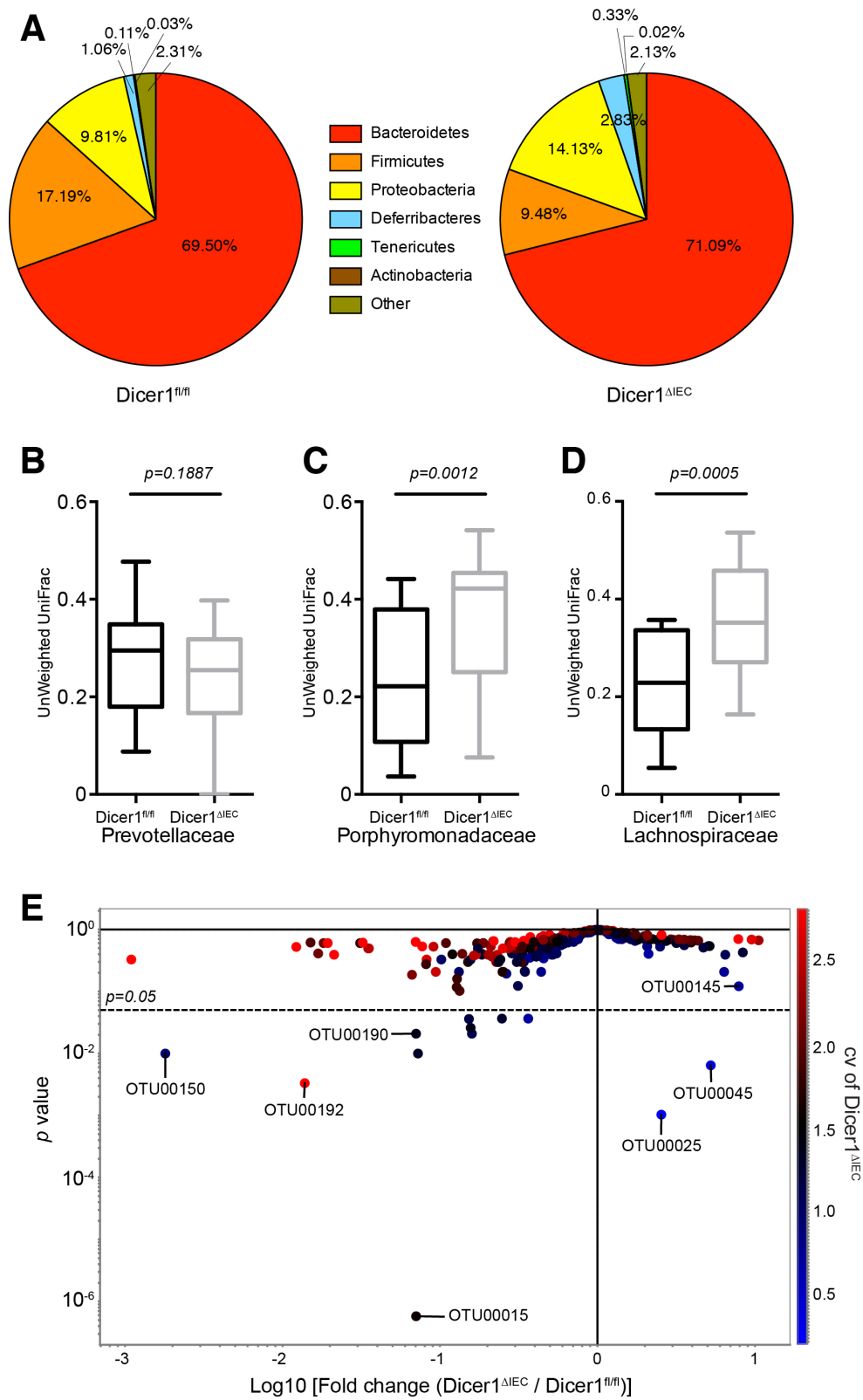Log10 [Fold change (Dicer1^ΔIEC / Dicer1^fl/fl)]

Figure S2.

Figure S2. Gut Microbial Community is Altered in the Intestinal Epithelial Cell Specific miRNA-deficient Mice

Related to Figure 3 and Figure 6.

Bacterial 16S rDNA sequence-based surveys were performed on the feces of 16 mice (n=7 Dicer1$^{fl/fl}$, 9 Dicer1$^{\Delta IEC}$ mice). (A) Pie graph represents the relative abundance of bacteria classified at a phylum-level taxonomy. (B to D) β-diversity at family level: (B) the bacterial family Prevotellaceae; (C) the bacterial family Porphyromonadaceae; (D) the bacterial family Lachnospiraceae. The specific distance metric used in each analysis is indicated on the axes. Values are: box: median, whiskers: min to max, *p*-value: non-parametric *t* test. (E) Volcano plots of sequencing based OTU reads identified between feces of Dicer1$^{fl/fl}$ and Dicer1$^{\Delta IEC}$ mice. Each dot represent one OTU; x-axis: base 10 logarithmic value of mean OTU reads ratio (Dicer1$^{\Delta IEC}$ vs. Dicer1$^{fl/fl}$); y-axis: *p* value comparing individual OTU between groups (unequal variance *t-test* with Benjamini-Hochberg correction); the color of the dot indicates coefficient of variation (cv) of the corresponding OTU in Dicer1$^{\Delta IEC}$ group as shown in side color scale bar. Tagged OTUs were checked in compensation experiments (Figure 6). Related to Table S5.
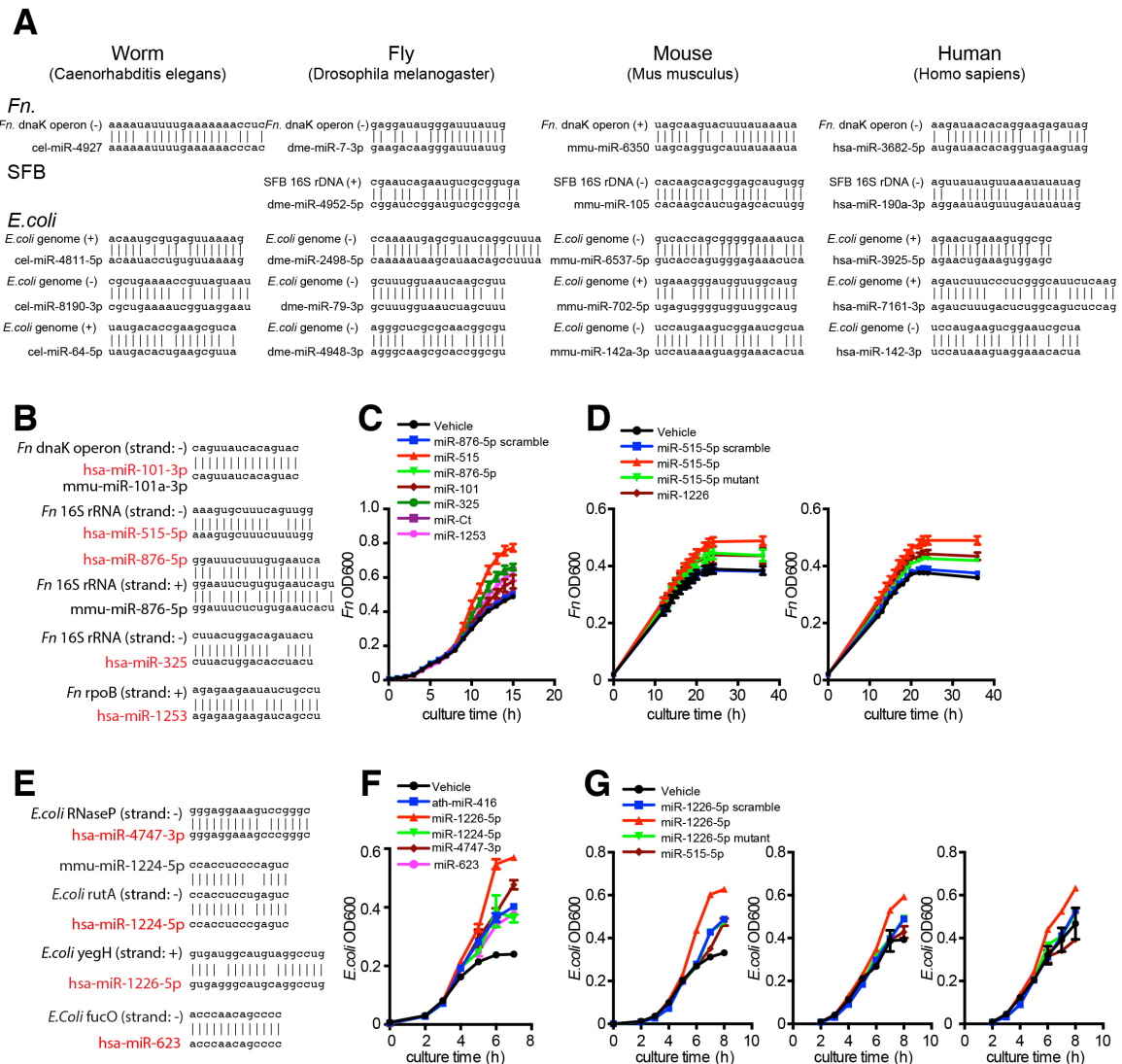
Figure S3.

Figure S3. Prediction of Host miRNAs Potential in Targeting Bacterial Genes and Their Effect on Bacterial Growth

Related to Figure 4.

(A) Bacterial gene sequences of *Fn*, SFB and *E. coli* were predicted by miRbase for potential miRNAs that target them. Example predicted hits alignment are listed with target strand sense (+)/ antisense (-) indicated. Details see also Table S6. (B-G) Sequence alignment of mouse and human miRNAs that were predicted to target bacterial dnaK

operon, 16S rRNA and rpoB of *Fn* (B); RNaseP, rutA, yegH and fucO of *E. coli* (E). The highlighted miRNAs were validated in a bacteria growth assay as shown in (C, D, F, G). C-D: Growth curve of *Fn* cultured in the presence of 1.25 μM of the indicated miRNA mimics; F-G: Growth curve of *E.coli* cultured in the presence of 2 μM of the indicated miRNA mimics. C and F: representative of two independent experiments. D and G: representatives of 5 independent experiments (see also Figure 4).
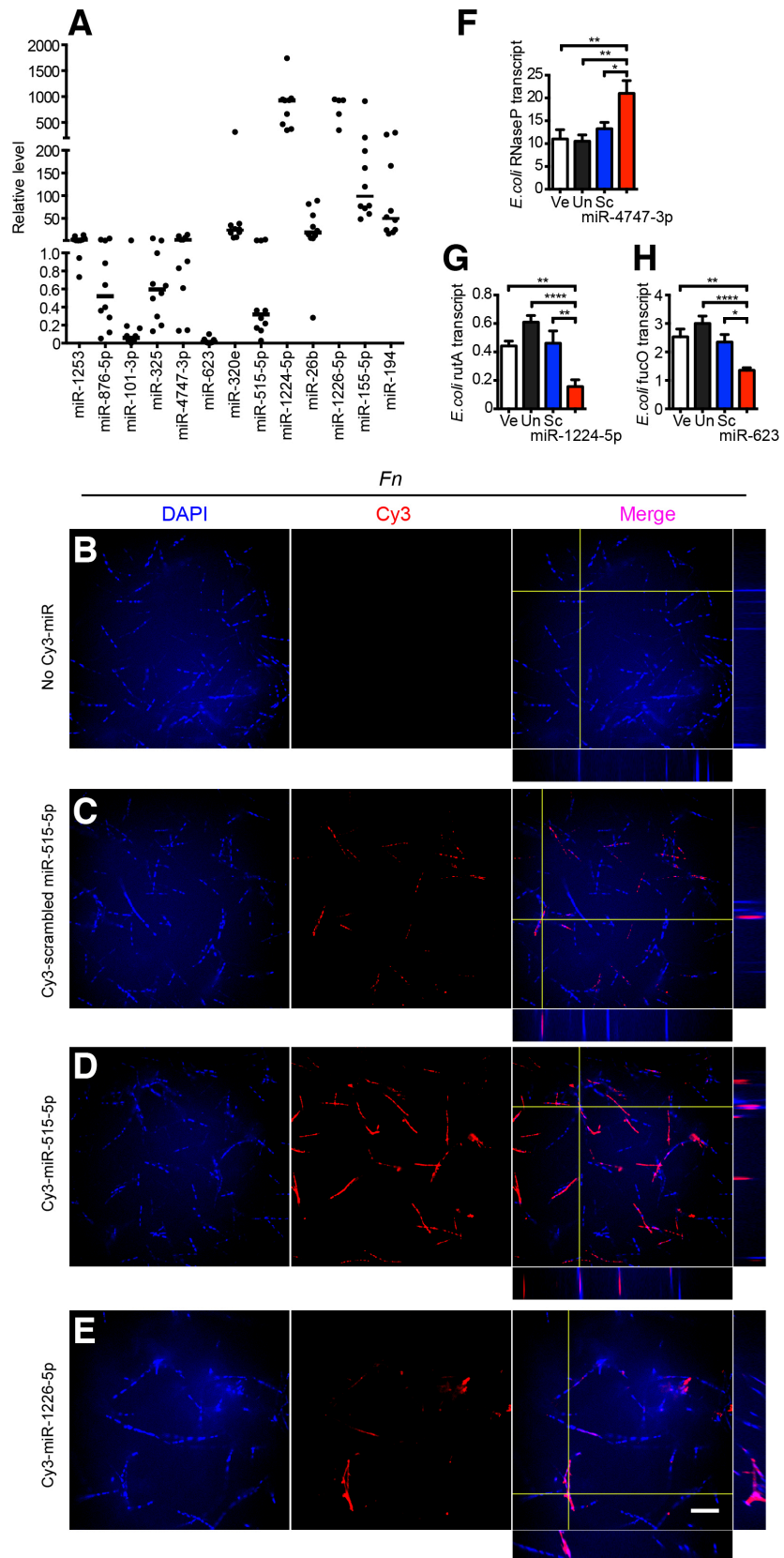
Figure S4.

Figure S4. MiRNA Effects on Bacterial Gene Transcript

Related to Figure 5

(A) The miRNAs that were used in bacteria culture (see Figure 4 and Figure 5), together with nanostring detected abundant miR-320e, miR-155-5p, miR-194 and miR-26b were examined for their abundance in human stool specimens by qPCR (reference: snoRNA135, data are individual value and median, n=10).

(B-E) *Fn* was cultured in the presence of 1.25 μM Cy3-labeled (red) hsa-miR-515-5p, scrambled hsa-miR-515-5p control or hsa-miR-1226-5p for 12 hours and washed with PBS, and fixed in 2% PFA, followed by nucleic acid staining with DAPI (Blue). Images were acquired by confocal microscopy with 100× objective. Merged channel and orthogonal view were processed with Fiji/ImageJ. Scale bars, 10 μm. Representative of 2 experiments. See also Movie S2.

(F-H) *E. coli* was cultured in the presence of vehicle (Ve), 2 μM miRNA mimics hsa-miR-4747-3p, hsa-miR-1224-5p, corresponding sequence-scrambled mimics (Sc) and sequence-unrelated ath-miR-416 (Un) as control for 2 hours. RNA was isolated and the transcript levels of *E. coli* RNaseP (F), rutA (G) and fucO (H) were quantified by qPCR reference to gyrA. Values are mean ± SEM, One-way ANOVA followed by Dunnett's multiple comparison tests. *p<0.05, **p<0.01, ****p<0.0001, data summarize 8 independent experiments.
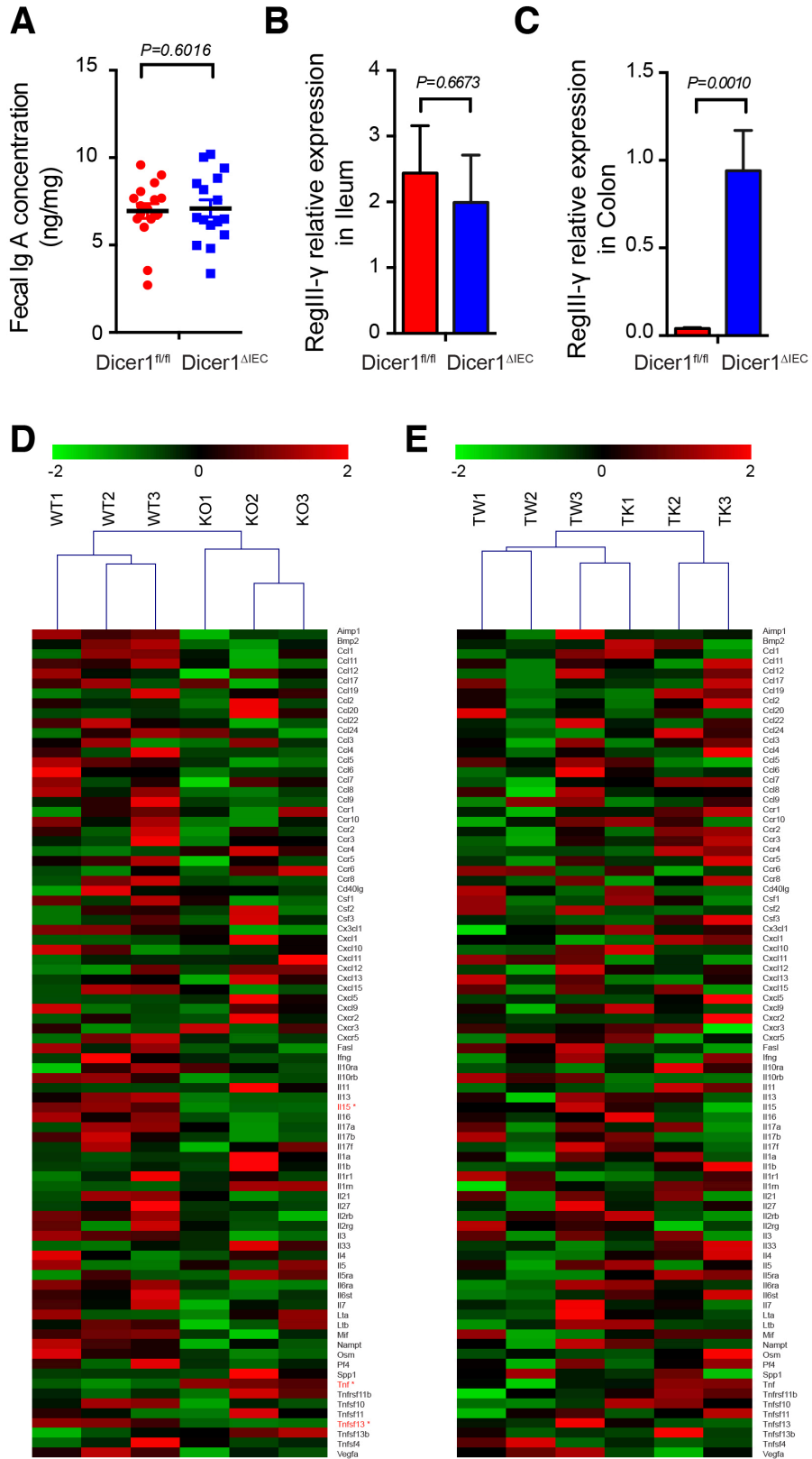
Figure S5.

Figure S5. Gut Anti-microbial Inflammatory Components in the Dicer1$^{\Delta IEC}$ Mice

Related to Figure 6

(A) Free IgA concentration in the feces of WT (n=17) and Dicer1$^{\Delta IEC}$ Mice (n=16) was determined by ELISA (Values are mean ± SEM, t-test).

(B and C) Relative RegIII-γ gene transcripts in the ileum (B) and colon (C) of WT and Dicer1$^{\Delta IEC}$ mice were quantified by qPCR (Values are mean ± SEM, n=10 mice per group, t-test).

(D-E) Three biological replicate RNA samples from colonic tissue of naïve WT, Dicer1$^{\Delta IEC}$ (KO), WT fecal RNA transplanted KO (TW) or KO fecal RNA transplanted KO (TK) mice were analyzed for inflammatory cytokines and receptors using PCR array. Heat maps show relative levels of each inflammatory cytokine and receptor and unsupervised hierarchical clustering from independent WT vs KO biological replicates (D), and TW vs TK biological replicates (E) (*: p<0.05, Benjamini-Hochberg corrected t-test).
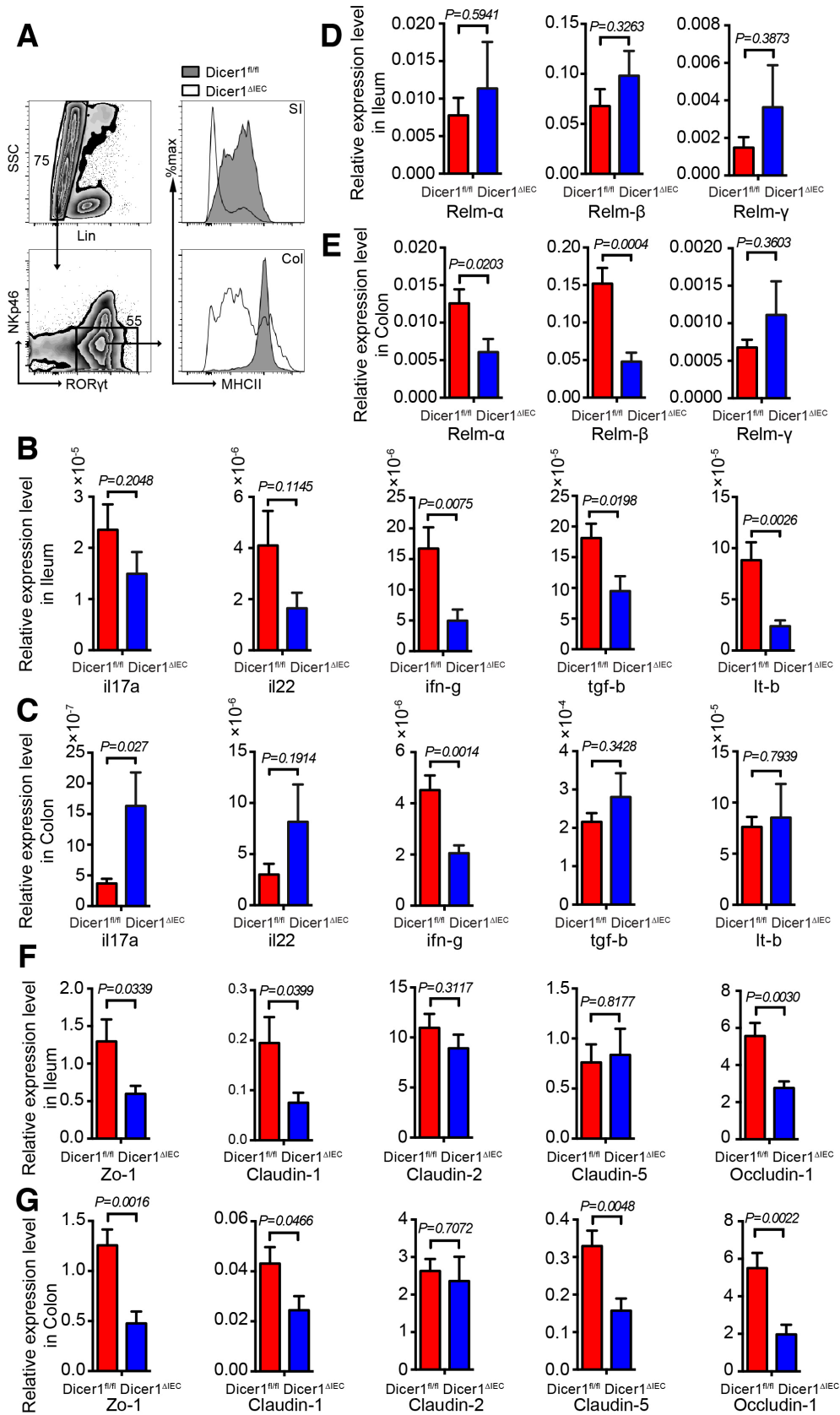
Figure S6.

Figure S6. Gut Epithelial miRNA-deficiency Alters Gut Innate Lymphoid Cells, Colonic Inflammatory and Barrier Function Molecules

Related to Figure 6.

(A) Naïve Dicer1$^{fl/fl}$ and Dicer1$^{\Delta IEC}$ littermates were examined for MHCII expression in the LTi cells by flow cytometry. Gating strategy for LTi cells and expression of MHCII in the LTi cells of small intestine (SI) and colon (Col) are shown. Data are representative of 3 independent experiments with 3 mice per group.

(B to G) RNA was isolated from Naïve Dicer1$^{fl/fl}$ and Dicer1$^{\Delta IEC}$ littermate mouse terminal ileum and colon tissue and relative transcripts of the following molecules were quantified by qPCR: (B and C) il-17a, il-22, ifn-γ, tgf-β and lt-β. (D and E) Resistin-like molecules (Relm-α, Relm-β, Relm-γ). (F and G) Tight junction protein transcripts. (Values are mean ± SEM, n ≥ 9 mice per group, t-test)

Table S1. Relative Level of miRNAs Detected in C57BL/6J Mouse Fecal Samples

Related to Figure 1.

6 C57BL/6J mouse fecal RNA samples were analyzed for miRNA using an nCounter mouse miRNA expression assay kit. Threshold was set to average of the negative control reads + 2× SD of negative control reads. The miRNAs tested by the assay and the mean reads of the positively detected miRNAs are shown.

Table S2. Relative Level of miRNAs Detected in Human Fecal Samples

Related to Figure 1.

10 human fecal RNA samples were analyzed for miRNA with an nCounter human miRNA expression assay kit. Threshold was set to average of the negative control reads + 2× SD of negative control reads. The miRNAs tested by the assay and the mean reads of the positively detected miRNAs are shown.

Table S3. Relative Level of miRNAs Detected in Dicer1$^{fl/fl}$ and Dicer1$^{\Delta IEC}$ Mouse Feces

Related to Figure 2.

RNA samples from Dicer1$^{fl/fl}$ ($n=5$) and Dicer1$^{\Delta IEC}$ ($n=6$) mouse fecal samples were analyzed for miRNA with an nCounter mouse miRNA expression assay kit. Threshold was set to average of the negative control reads + 2× SD of the negative control reads. The mean reads of the positively detected miRNAs of both groups are shown.

Table S4. Relative Level of miRNAs Detected in Dicer1$^{fl/fl}$ and Dicer1$^{\Delta Hopx}$ Mouse Feces

Related to Figure 2.

Fecal RNA samples isolated from tamoxifen treated Dicer1$^{fl/fl}$ ($n=4$) and Dicer1$^{\Delta Hopx}$ ($n=4$) mice were analyzed for miRNA with an nCounter mouse miRNA expression assay kit. Threshold was set to average of the negative control reads + 2× SD of the negative control reads. The mean reads of the positively detected miRNAs of both groups are shown.

Table S5. Fecal Microbiota OTU Abundance in Dicer1$^{fl/fl}$ and Dicer1$^{\Delta IEC}$ Mice

Related to Figure 3 and Figure S2.

Bacterial 16S rDNA sequence-based surveys were performed on the feces of 16 mice (n=7 Dicer1$^{fl/fl}$, 9 Dicer1$^{\Delta IEC}$ mice). OTUs with mean counts >2 are shown.

Table S6. MiRNAs from a Broad Range of Species Are Predicted to Target Bacterial Genes

Related to Figure 4 and Figure S3.

Example of prediction for miRNAs targeting bacterial sequences: nine *Fn*, SFB and *E. coli* nucleic acid sequences were predicted for miRNAs that could potentially target the sequences by aligning the sequences using miRbase.org online tool. The table lists miR hits from a broad range of species.

Movie S1 and Movie S2. Host miRNA Enters Bacteria and Co-localizes with Bacterial Nucleic Acids

Related to Figures 5A-5E and Figures S4B-S4E

E. coli GFP (Green) were cultured in the presence of Cy3-labeled hsa-miR-1226-5p (Red) for 4 hours (Movie S1) and *Fn* were cultured in the presence of Cy3-labled hsa-miR-515-5p (Red) for 12 hours (Movie S2), washed with PBS and fixed with PFA, followed by nucleic acids staining with DAPI (Blue). Z-stack images were acquired by confocal microscopy. Merged channels of *E. coli* (Movie S1) and *Fn* (Movie S2) were processed with Fiji/ImageJ. Representative of 2 experiments.

EXTENDED EXPERIMENTAL PROCEDURES

Animals, Tissue and Feces Sampling

Animal procedures were approved by the Harvard Medical Area (HMA) Standing Committee on Animals. C57BL/6J (stock number 000664), B6.Cg-Tg(Vil-cre)997Gum/J (Van Niel et al., 2003) (Vil-cre, stock number 004586), $Hopx^{tm2.1(cre/ERT2)Joe}$/J (Takeda et al., 2011; Van Niel et al., 2003) (Hopx-cre, stock number 017606), B6.Cg-Dicer1^{tm1Bdh}/J (Harfe et al., 2005) (Dicer1-flox, stock number 006366), and B6.129S7-Rag1^{tm1Mom}/J (Mombaerts et al., 1992) (stock number 002216) mice were from The Jackson Laboratory. Mice with intestinal epithelial cell specific deletion of Dicer1 were generated by cross-breeding Vil-cre and Dicer1-flox mice. Vil-cre$^{+/-}$-Dicer1$^{fl/fl}$ mice were compared to Vil-cre$^{-/-}$-Dicer1$^{fl/fl}$ littermates. Mice with Hopx-expressing cell specific Dicer1 depletion were generated by cross-breeding Hopx-cre and Dicer1-flox mice. Hopx-cre$^{+/-}$-Dicer1$^{fl/fl}$ mice were compared to Hopx-cre$^{-/-}$-Dicer1$^{fl/fl}$ littermates three days after a period of five consecutive days of 1 mg/day tamoxifen (catalog number: T5648, Sigma-Aldrich) intraperitonealy injection (Feil et al., 2009). Otherwise specified, all the mice used in the experiments were 6-8 wks old. All mice were housed under specific pathogen-free (SPF) conditions. For the germ-free conventionalization assay, germ-free SWGF mice (Taconic farm) were maintained in sterile isolators on a commercial laboratory chow diet. Conventionalization was performed by oral gavage of freshly obtained caecal material from SPF raised C57BL/6J mice; animals were used for experiments seven days thereafter. Fecal specimens and intestinal luminal contents were collected, snap frozen, and stored at -80 °C for microbiota or miRNA analysis. To analyze gut tissue gene transcripts, 0.5 cm of tissue from distal small intestinal segments

(ileum) and 0.5 cm of tissue from proximal colon were collected by removing luminal contents, washed with sterile PBS and stored at -80 ℃ until analysis.

Antibiotic Treatment

Mice were given a mixture of antibiotics (ampicillin 1 mg/ml, vancomycin 500 mg/ml, neomycin 1 mg/ml, metronidazole 1 mg/ml, and streptomycin 1 mg/ml (Sigma-Aldrich)) in drinking water *ad libitum* for 1 week following an established protocol (Benjamin et al., 2013). The removal of bacteria was confirmed by culture the colonic luminal content anaerobically on BHI agar containing 10% calf blood and aerobically on LB agar.

Human Fecal Samples

Human fecal specimens were collected from 10 healthy subjects (7 females). All subjects gave written consent according to a protocol approved by the Institutional Review Board at Brigham and Women's Hospital. Healthy volunteers were excluded for GI disorders, antibiotics, or probiotic use in the last 2 months and during the sampling period. Subjects were between 24-59 years old. All stool samples were collected using Commode specimen collection system (Fisher Scientific) and stored at -80℃ until further processing.

Fecal RNA Isolation

Total RNA (including miRNAs) was extracted from stool specimens using mirVana™ miRNA isolation kit (catalog number: AM1560, Ambion®) following the manufacturer's procedure for total RNA isolation with modifications. Briefly, 60 mg of mouse or 200 mg

of human stool was homogenized in 600 μl sterile PBS. RNA was extracted with acid Acid-Phenol: Chloroform extraction. Aqueous phase precipitation was performed by mixing with 1.25 volumes of 100% ethanol, followed by purification on a glass fiber filter cartridge. Following elution, RNA quality was assessed by A260/A280 ratios using ND-1000 nanodrop. Further purification was performed using Amicon® Ultra-0.5 Centrifugal Filter Devices-3k (Ultracel-3 K membrane, catalogue number: UFC500396, Millipore) to obtain the RNA quality of A260/A280: 1.8~2.0, A260/A230: ≥1.3, no detectable bacterial RNA by qPCR. Samples were stored at -80 ℃ until analysis.

RNA Isolation from Tissue

Total RNA from spleen and small intestine tissue was extracted using mirVana™ miRNA isolation kit (catalog number: AM1560, Ambion®) following the manufacturer's protocol.

RNA Detection by Bioanalyzer

200 ng of total RNA isolates from spleen cells and fecal samples were analyzed using Agilent 2100 Bioanalyzer (http://www.chem.agilent.com) with an RNA 6000 Nano Kit for total RNA and Agilent small RNA Kit for small RNA. The electropherograms were analyzed using the Agilent 2100 Expert Bioanalyzer Software.

Quantitative NanoString nCounter Fecal miRNA Analysis

Nanostring nCounter technology (http://www.nanostring.com/) allows expression analysis of multiple genes from a single sample (Butovsky et al., 2014). nCounter®

mouse miRNA Assay Kit and nCounter® human miRNA Assay Kit (NanoString Technologies) were used to detect miRNA in fecal RNA isolates. Identical amount of 300 ng total RNA per sample was loaded in nCounter analysis following the manufacturer's protocol. Data were analyzed with nSolver™ Analysis Software1.1 with normalization to assay and spike-in controls and visualized with the Multiplot studio v1.0 module from GenePattern (Li et al., 2014; Reich et al., 2006) for volcano plotting and with JMP11 soft for Principal component analysis (PCA).

MiRNA Detection with qPCR

qPCR was performed to detect miRNAs that were identified in NanoString nCounter assay using TaqMan® MiRNA Reverse Transcription (Applied Biosystems) and Taqman Universal PCR Master Mix according to the manufacturer's protocol. The input of fecal RNA per sample was 20 ng. The TaqMan® MiRNA Assay (Applied Biosystems) IDs for corresponding miRNA were: snoRNA135: 001230 (reference); mmu-miR-1224: 240985_mat; mmu-miR-155: 002571; mmu-miR-194-5p: 000493; mmu-miR-26b-5p: 000407; hsa-miR-1253: 002894; hsa-miR-876-5p: 002205; hsa-miR-101-3p: 002253; hsa-miR-325: 000540; hsa-miR-515-5p: 001112; hsa-miR-4747-3p: 462151_mat; hsa-miR-320e: 243005_mat; hsa-miR-194: 000493; hsa-miR-26b: 000406; hsa-miR-1226-5p: 002758; hsa-miR-623: 001555.

Fecal Suspension Particle size Distribution by NanoSight and EM

Feces from 6 week-old female germ-free SWGF mice were suspended in PBS to 30 mg/ml, spun down at 10,000 ×g for 5 min to remove debris and then filtered through a

0.8 μm filter, the flow through was 1/500 diluted and then applied to the NanoSight LM10 system. The 0.8 μm filtrates were further filtered with a 0.2 μm filter and the filtrates were observed by electron microscopy.

Fecal Extracellular Vesicle Isolation and RNA extraction

Feces from germ-free SWGF mouse were homogenized in PBS, spun down at 300 ×g for 10 min. The supernatant was further spun down at 2000 ×g for 15 min and supernatant was filtered through a 0.8 μm filter (Millipore). Extracellular vesicles were isolated from the filtrate using exoEasy Maxi Spin Columns (Qiagen), followed by the RNA isolation using miRCURY RNA isolation kit (Exiqon) with on-column DNase treatment (Qiagen).

Fecal Microbes Quantification by qPCR

DNA was extracted from fecal pellets using a QIAamp Fast DNA Stool Mini Kit (Qiagen). qPCR analysis was conducted using a ViiA7 system (Applied Biosystems). SFB, *Fn, E.coli, B.fragilis* and OTUs quantification was conducted by Taqman amplification reactions consisting of genomic DNA, TaqMan® Universal PCR Master Mix (Applied Biosystems), and primer pairs as follows: All bacteria (reference): Bact_8F: AGAGTTTGATCMTGGCTCAG,Bact_515R:

TTACCGCGGCKGCTGGCAC, probe: CCAKACTCCTACGGGAGGCAGCAG (Dridi et al., 2009); SFB: 16S358F: ATATTGCACAATGGGGGAAA, 16S459R: GGTACCGTCCTTCCTCTTCC, probe: CGCGTGAGTGAAGAAGGTTT (Dridi et al., 2009). *Fn*: Forward: CAGGATGAACGCTGACAGAA, Reverse: TAATGGGACGCAAAGCTCTC, Probe: AGGGACAACATTTGGAAACG. *E.coli:*

Forward: AGGCCTTCGGGTTGTAAAGT, Reverse: CGGGGATTTCACATCTGACT, Probe: CAGAAGAAGCACCGGCTAAC. *B. fragilis*: 16S127F: CGTATCCAACCTGCCCTTTA, 16S317R: GGGGACCTTCCTCTCAGAAC, Probe: AGGATTCCGGTAAAGGATGG. OTU00389: Forward: AAAGGGAGCGTAGGTGGATT Reverse: CTTCGCAATCGGAGTTCTTC, Probe: TGCAGTTGAAACTGGCAGTC. OTU00015: Forward: TGAAACTGCCGTGCTAGAGA, Reverse: CTGTTCGATACCCACGCTTT, Probe: ATATTGCGCAGAACTCCGAT. OTU00025: Forward: AGGCGGAATAGCAAGTCAGA, Reverse: GTCGCCTTCGCAATGAGTAT, Probe: TGGAATTCTTGGTGTAGGGG. OTU00045: Forward: TTACTGGGCGTAAAGGGTGT, Reverse: CCATCGGTGTTCTTCCAAAT, Probe: ACCTGGGAAAGCCATACATG. OTU00192: TGTCCGGATTTACTGGGTGT, Reverse: CTACGCATTTCACCGCTACA, Probe: AGTCAGATGTGAAATCCCGC. OTU00190: Forward: TGGGTTTAAAGGGTGCGTAG, Reverse: ATGCATTTCACCGCTACACC, Probe: GATCTTGAGTGGGCGAGAAG. OTU00150: Forward: ACTCGGGTGCTGCTTCTAAA, Reverse: CTGTTTGCTCCCCACACTTT, Probe: GCGGTGGAATGCGTAGATAT. The relative quantity of 16S rDNA for each bacterial group was calculated by the ΔCt method and normalized to the amount of all bacteria in the sample.

16S rRNA Gene Analyses

Fecal DNA was isolated as described above. Amplicons spanning variable region 4 (V4) of the 16S rRNA gene were generated with primer containing barcodes using

NEXTflex™ 16S V4 Amplicon-Seq Kit (Bioscientific) and sequenced on illumina miSeq. Data was processed using the QIIME software (v1.9.0) following an established protocol (Caporaso et al., 2010). Briefly, >250,000 total sequences were generated for each mouse sample. Reads were assigned to 1895 OTUs using a closed-reference OTU picking protocol using the QIIME filtered at 97% identity. Following this, a phylogenetic tree was constructed from the representative sequences. The rarefied OTU frequency matrix (subsampled using mothur) and phylogenetic tree were used with QIIME's beta diversity analysis, which produced weighted and unweighted UniFrac, and bray-curtis distance matrices for each comparison. A Principal coordinates analysis (PCoA) plot was generated based on Unifrac distances matrices, which were used to test the similarity of the community members.

Bacterial Strain and Growth Conditions

Fusobacterium nucleatum (*Fn*) ATCC® 10953™ was grown anaerobically on Trypticase Soy Agar with 5% Sheep Blood (TSA II) for colony isolation and in basal medium (Oxoid) (Pantosti et al., 1991) for growth measurements. *E.coli* ATCC® 47016™ and *E.coli* GFP (ATCC 25922GFP) were grown aerobically on LB agar for colony isolation and in LB medium for growth assays.

miRNA Target Prediction

Nucleic acid sequences of *Fn* polymorphum dnaK operon (AJ512795.1), *Fn* 16S ribosomal RNA and 23S ribosomal RNA gene (AF342832.1 and NR_041807.1), *Fn* DNA-directed RNA polymerase beta chain (rpoB) gene (GQ274957.1), SFB 16S rDNA

(gi: 457480), *E.coli* genome (gi:26111730), *E.coli* gene for RNA component of RNaseP (D90212.1), *E. coli* Lactaldehyde reductase fucO (gb:AE014075.1), *E.coli* pyrimidine monooxygenase RutA (gi:639610644) were predicted for miRNAs that could potentially target the sequence by aligning with online miRBase search (http://mirbase.org) (Griffiths-Jones et al., 2008).

In vitro Bacteria Growth Measurements

The anaerobic bacteria *Fn* were cultured at 37ºC by inoculating 2 ml aliquots of anaerobe basal medium with bacteria colony from a fresh plate and grown anaerobically in an anaerobic chamber. These cultures were used to inoculate aliquots of basal medium (1/50), with inoculum volumes of 150 μl per well in microtiter plates adjusted to contain same number of viable cells based on previous measurements. Mission® miRNA mimics hsa-miR-876-5p (sequence: UGGAUUUCUUUGUGAAUCACCA), scrambled hsa-miR-876-5p (Sequence: GUGUUCCGACGUAUCUAUUAAU) hsa-miR-101-3p (sequence: UACAGUACUGUGAUAACUGAA), hsa-miR-1253 (sequence: AGAGAAGAAGAUCAGCCUGCA), hsa-miR-325 (sequence: CCUAGUAGGUGUCCAGUAAGUGU), hsa-miR-515-5p (sequence: UUCUCCAAAAGAAAGCACUUUCUG), scrambled hsa-miR-515-5p (AUACAGUACGACUUCACGAUCUAU), mutated hsa-miR-515-5p (UUCUCAACAAGACGUCGCGUCCUG), and Negative Control mimic (miRNA NegCt, miR-Con) (sequence: GGUUCGUACGUACACUGUUCA, ath-miR-416) from Sigma-Aldrich were supplied in the culture at the concentration of 1.25 μM. Growth was

monitored as absorbance at 600 nm (OD_{600}) once per hour for up to 36 hours with a spectrophotometer (Infinite® F200, Tecan).

E.coli was cultured by 2 ml aliquots of LB medium with bacteria colony from a fresh plate and grown aerobically to log phase. These cultures were used to inoculate aliquots of LB medium (1/50), with inoculum volumes of 150 μl per well on a 96-well plate adjusted to contain same number of viable cells based on previous measurements and cultured at 37ºC with 250 rpm shaking. Mission® miRNA mimic hsa-miR-1224-5p (sequence: GUGAGGACUCGGGAGGUGG), hsa-miR-4747-3p (sequence: AAGGCCCGGGCUUUCCUCCCAG), hsa-miR-4747-3p scramble (sequence: GGCGCAUUACUCGCGACUCGCC), hsa-miR-623 (sequence: AUCCCUUGCAGGGGCUGUUGGGU), hsa-miR-623 scramble (sequence: GCUUGGUCGCGUUAGGCGGCTAU), hsa-miR-1226-5p (sequence: GUGAGGGCAUGCAGGCCUGGAUGGGG), hsa-miR-1226-5p scramble (sequence: GGGUGAGGGAGGUCGGCUGGGUACCA), mutated hsa-miR-1226-5p (sequence: GUCAGGCCAUCCAGGGGUGGUUGGGG) and Negative control mimic (miR NegCt, miR-Con) (sequence: GGUUCGUACGUACACUGUUCA, ath-miR-416) from Sigma-Aldrich were supplied in the culture at the concentration of 2 μM. Growth was monitored as absorbance at 600 nm (OD_{600}) once per hour for up to 8 hours with a spectrophotometer.

Detection of miRNA Entering Bacteria by Confocal and Flow Cytometry

E.coli GFP was aerobically cultured at 37ºC with 250 rpm shaking for 0, 5 min, 2 hours and 4 hours (Confocal only take 4 hours point) in the presence of 2 μM Cy3-labeled hsa-

miR-1226-5p (sequence see above), scrambled hsa-miR-1226-5p (sequence see above) and Cy3 labeled hsa-miR-515-5p (sequence see above) (GE dharmacon). *Fn* was anaerobically cultured in anaerobic chamber at 37°C for 0, 5 min, 6 hours, 12 hours (Confocal only take 12 hours point) in the presence of 1.25 μM Cy3-labeled hsa-miR-515-5p (sequence see above), scrambled hsa-miR-515-5p (sequence see above) and Cy3 labeled hsa-miR-1226-5p (sequence see above) (GE dharmacon). All bacteria were terminated by washing with ice cold PBS and fixed with ice cold 2% PFA and then acquired with the BD™ LSR II Flow Cytometry System (BD Biosciences); and data were analyzed with FlowJo Software (TreeStar); For confocal observation, samples were followed by nucleic acids staining with DAPI, visualized with a 100× objective and z-stack images were captured with Yokogawa Spinning disk confocal microscope system. Images were processed with Fiji/ImageJ for channels merging, orthogonal view and reslice.

Fecal Free IgA Quantification

Fresh fecal pellets were collected and resuspended at 10 mg/ml in PBS. Mouse IgA in the suspension was determined with a mouse IgA ELISA kit (ALP) (Mabtech) following the manufacturer's protocol.

Fecal RNA Transplantation and Fecal miRNA Fn Maintenance Assay

RNA was isolated from freshly collected feces from 6-8 weeks old gender matched Dicer1$^{\Delta IEC}$ and WT littermates using mirVana™ miRNA isolation kit as described above. The fecal RNA eluted in nuclease free water was administrated to 6-weeks old Dicer1$^{\Delta IEC}$

littermates by gavage at the dosage of 22.5 μg/day for 7 consecutive days. Feces of recipient mice were collected at day 7 for microbes profiling by qPCR. The recipient mice were then treated with DSS for colitis induction.

In a pilot experiment Fn was not detected in C57BL/6J mice in our facility. To investigate whether different fecal miRNA settings differ in supporting Fn growth in $vivo$, Fn was anaerobically cultured to an exponential growth phase of OD_{600}=0.2. 200 μl of this culture was given to Dicer1$^{fl/fl}$ and Dicer1$^{\Delta IEC}$ mice by gavage. Fn abundance in the mouse feces was monitored by qPCR at Day 0, Day 1, Day 2, Day 3 and Day 6.

In order to investigate the role of specific mciroRNA on specific bacteria in vivo, synthesized miRNA mimics hsa-miR-1226-5p, hsa-miR-4747-3p, and miRNA control (ath-miR-416) were fed to C57BL/6J mice continuously at a concentration of 200 nM in drinking water for 48 hours. Feces were collected for $E.coli$ abundance detection by qPCR.

Bacterial Gene Transcript Quantification by qPCR

Fn was cultured in the presence of Vehicle, 1.25 μM miRNA mimics hsa-miR-515-5p, scrambled hsa-miR-515-5p mimics and mutated hsa-miR-515-5p mimics, as well as hsa-miR-1226-5p for 16 hours. $E.coli$ was cultured in the presence of miRNA mimics hsa-miR-1226-5p, scrambled hsa-miR-1226-5p mimics and mutated hsa-miR-1226-5p mimics, as well as hsa-miR-515-5p at the concentration of 2 μM for 4 hours and stopped by chilled on ice and stabilized with RNAlater® Solutions (Ambion). Total bacterial RNA from cultured bacterial cells was extracted using TRIzol® Max™ Bacterial RNA isolation Kit (Ambion) following the manufacturer's protocol. cDNA was made using

High Capacity cDNA Reverse Transcription Kit (Applied biosystems). qPCR was performed using Taqman Universal PCR Master Mix and TaqMan® Gene Expression Assay primer pairs as following: *Fn*: 23S rRNA: Forward: GAGCCGATAAGGACGTGGTA, Reverse: TTTAGGCTTGACCCATTTCG, Probe: AATACGAAAGAGGGAACCGC (as reference for 16S rRNA, not predicted to be targeted by any miRNA determined for transcripts in this study). *E.coli:* gyrA gene (as reference gene, not predicted to be targeted by any miRNA tested in this study): Forward: TTCTTCTTCTGGCTCGTCGT, Reverse: GTTTCGGAAATCAGCATCGT, Probe: GACCCACCACGTTTTCATCT; RNaseP: Forward: CCAGTGCAACAGAGAGCAAA Reverse: TCATTCATCTAGGCCAGCAA, Probe: TCATAAGGTACGGCCCGTAC; rutA: Forward: TGAGTCAGCGCAGGTAGATG, Reverse: CTTCGGCGGTAAACATCAAT, Probe: TCGATTCCCGACAGAAAATC. The relative quantity of each bacterial gene transcripts was calculated by the ΔCt method and normalized to the amount of reference gene of the sample.

Flow Cytometry

Intraepithelial lymphocytes in the colon and small intestine of Dicer1$^{fl/fl}$ and Dicer1$^{\Delta IEC}$ mice were isolated using a Lamina Propria Dissociation Kit (Miltenyi Biotec) followed by separation through Percoll (GE Healthcare Life Sciences) gradient centrifugation. For flow cytometry analyses of MHCII expression in LTi cells, cells were pre-blocked with blocking antibody to CD16/CD32 Fc (clone 2.4G2, BD Biosciences) and stained with antibodies to the following markers: anti-Lin, combinations of mouse Lineage antibody cocktail-PB (Components include anti-mouse CD3, clone 17A2; anti-mouse Ly-6G/Ly-

6C, clone RB6-8C5; anti-mouse CD11b, clone M1/70; anti-mouse CD45R/B220, clone RA3-6B2; anti-mouse TER-119/Erythroid cells, clone Ter-119, BioLegend), anti-NKp46-APC (clone 29A1.4, Biolegend), anti-MHCII-PerCP-eFluor®710 (clone M5/114.15.2, eBioscience). For intracellular staining, cells were fixed and permeabilized using a commercially available kit (eBioscience) and stained with anti-RORγt-PE (clone AFKJS-9, eBioscience). Gating strategy for LTi cells was Lin$^-$RORγt$^+$NKp46$^-$ (Hepworth et al., 2013; Walker et al., 2013). Analysis was performed on a BD™ LSR II Flow Cytometer System (BD Biosciences), and data was analyzed with FlowJo Software (TreeStar).

To compare the miRNA profile in gut epithelial cells vs. gut luminal content, colon cells were first isolated by digesting colon tissue with buffer containing 5 mM EDTA, 5% fetal bovine serum (FBS) and 1 mM DTT; and then epithelial cells were sorted out with EpCAM antibody (Biolegend).

qPCR of Intestinal Tissue

To test cytokine levels in the intestine, RNA from distal ileum and proximal colon tissue was isolated using mirVana™ miRNA isolation kit. cDNA was made using High Capacity cDNA Reverse Transcription Kit (Applied biosystems). qPCR was performed using Taqman Universal PCR Master Mix and TaqMan® Gene Expression Assays: gapdh assay ID:Mm99999915_g1 (reference gene); il17a assay ID:Mm00439618_m1; il22 assay ID:Mm01226722_g1; ifng assay ID:Mm01168134_m1; tnf-a assay ID: Mm01161290_g1; tgfb1 assay ID: Mm01178820_m1; LTb assay ID: Mm00434774_g1. Reg3g assay ID: Mm01181783_g1; Relm-a assay ID: Mm00445109_m1; Relm-b assay

ID: Mm00445845_m1; Relm-g assay: Mm00731489_s1. Tight junction protein (Zo-1, claudin-1, claudin-2, claudin-5, occluding-1) transcripts were determined by SYBR green approach using SYBR® Green PCR Master mix (Applied Biosystems) and published primers (Liu et al., 2013)

To quantify the transcriptional expression of inflammatory cytokines and receptors in the mice, RT2 Profiler PCR Arrays and RT^2 SYBR® Green qPCR Mastermix (Qiagen) was used following the protocol provided by the manufacturer.

DSS Colitis

6-week old Vil-cre$^{+/-}$ -Dicer1$^{fl/fl}$ mice and Vil-cre$^{-/-}$-Dicer1$^{fl/fl}$ littermates, or C57BL/6J Mice were treated with 3% Dextran sodium sulfate (DSS, Molecular Weight 36,000-50,000, Catalog number: 160110, MP Biomedicals) in the drinking water *ad libitum* for 7 days to induce colitis. In miRNA source checking experiment, fecal specimens were collected 4 days after DSS treatment.

Histology

Formalin-fixed colon Swiss roll was embedded in paraffin and 10 μm sections were cut and stained with haematoxylin and eosin.

Statistical Analysis

Unless otherwise indicated, data were analyzed with GraphPad Prism 6.0c software (San Diego, CA, USA). The differences between two groups were analyzed with Student's t-test with proper correction. The differences between more than two groups were analyzed

using ANOVA with appropriate multiple comparisons test. A two-sided p-value of <0.05 was regarded as significant. Except for specified in the context, results are expressed as mean ± SEM

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