

Figure S1. Bacterial regulation of *Rdh7* expression in liver and colon. Related to Figure 2

(A) mRNA quantification of genes involved in Vitamin A metabolism in liver of conventional (CV) and germ-free (GF). (B) mRNA quantification of *Lrat* in liver tissue of conventional (CV) and germ-free (GF). Anti-Rdh7 staining of colon tissues of conventional (CV) and germ-free (GF) mice. qPCR analysis of *Rdh7* mRNA in colon tissues of conventional (CV) and germ-free (GF) mice.

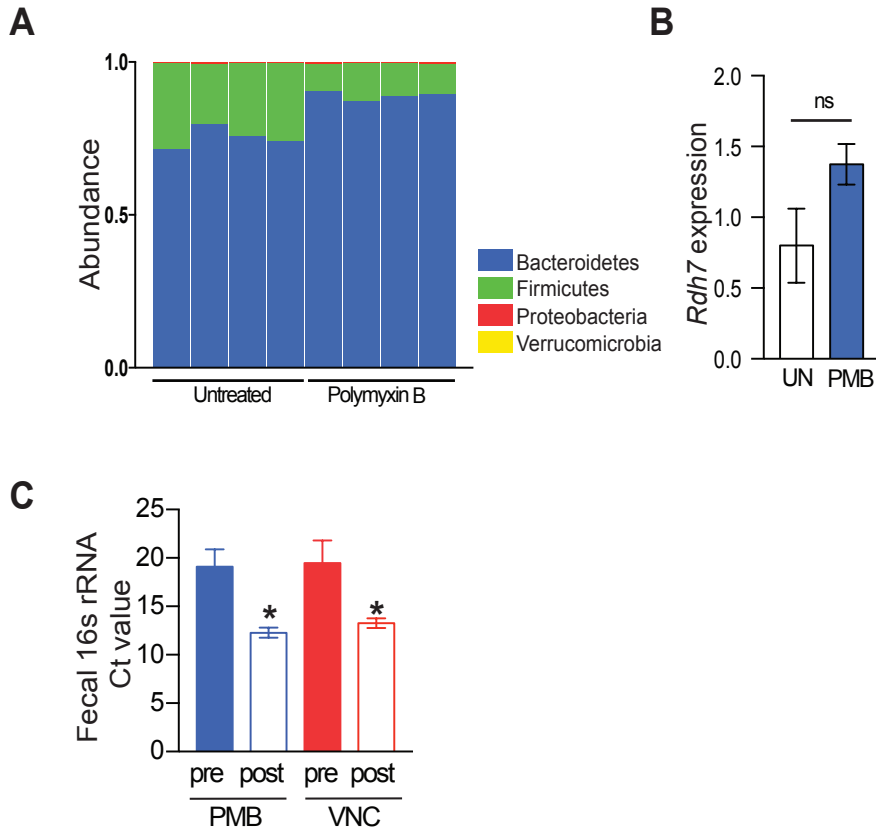


Figure S2. Effect of antibiotics on gut microbiota and *Rdh7* expression. Related to Figure 3

(A) Relative abundance of bacteria at phylum level determined by 16s rRNA analysis of fecal microbiome from untreated and polymyxin B treated mice. Mice were treated with polymyxin B (1000mg/L) in drinking water for four weeks. (B) *Rdh7* mRNA quantification by qPCR in colon tissue mice treated with untreated versus polymyxin B treated mice. (C) 16s qPCR to look at bacteria numbers in mice treated with vancomycin and polymyxin B. Figures A-C are representative figures of a single experiment that was done 3 individual times. Student's t-test. Error bars represent SEM. * $P < 0.05$.

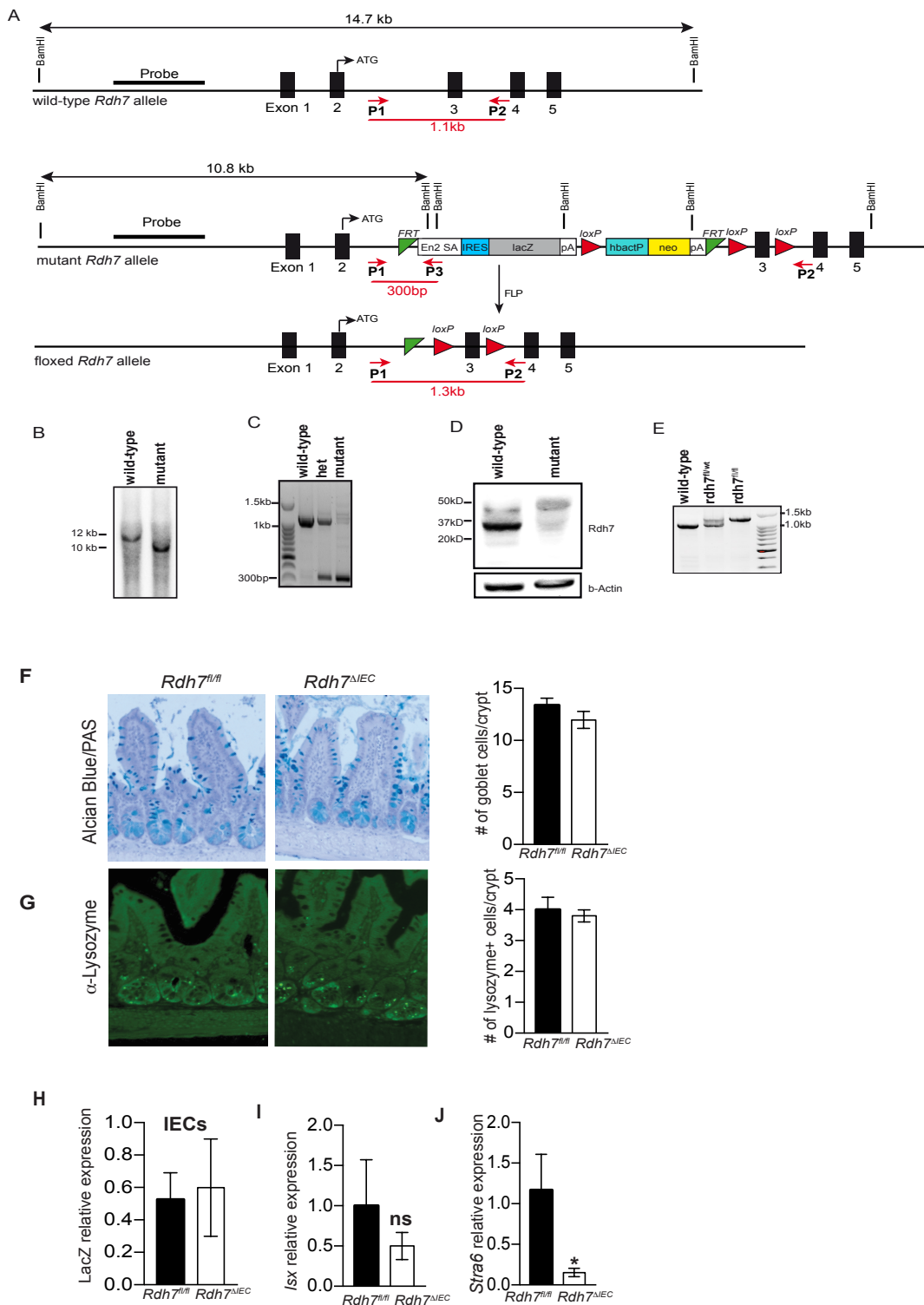


Figure S3. Generation and validation of *Rdh7^{fl/fl}* and *Rdh7^{ΔIEC}* mouse models. Related to Figure 4

(A) Scheme for generation of *Rdh7^{fl/fl}* and *Rdh7^{ΔIEC}* mouse models (B) Southern Blot confirming correct insertion of trapping vector. (C) PCR results confirming correct insertion. (D) Western Blot results testing for *Rdh7* in *Rdh7^{fl/fl}* and *Rdh7^{ΔIEC}*. (E) Genotyping results for *Rdh7^{fl/fl}* and *Rdh7^{ΔIEC}*. (F) Alcian Blue staining in paraffin embedded colon tissue to look at goblet cell numbers in *Rdh7^{fl/fl}* and *Rdh7^{ΔIEC}* mice. Graph on the side shows quantification of goblet cells from Alcian Blue staining. (G) Lysozyme staining in paraffin embedded colon tissue to look at Paneth cell numbers in *Rdh7^{fl/fl}* and *Rdh7^{ΔIEC}* mice. Graph on the side shows quantification of Paneth cells from Lysozyme staining. (H) *LacZ* mRNA quantification in intestinal epithelial cells (IECs) from *Rdh7^{fl/fl}* and *Rdh7^{ΔIEC}* (I) *Isx* mRNA quantification in intestinal tissue from *Rdh7^{fl/fl}* and *Rdh7^{ΔIEC}*. (J) *Stra6* mRNA quantification in intestinal tissue from *Rdh7^{fl/fl}* and *Rdh7^{ΔIEC}*. For figures F, G, H-J Student's t-test was performed, Error bars represent SEM. **P* < .05.

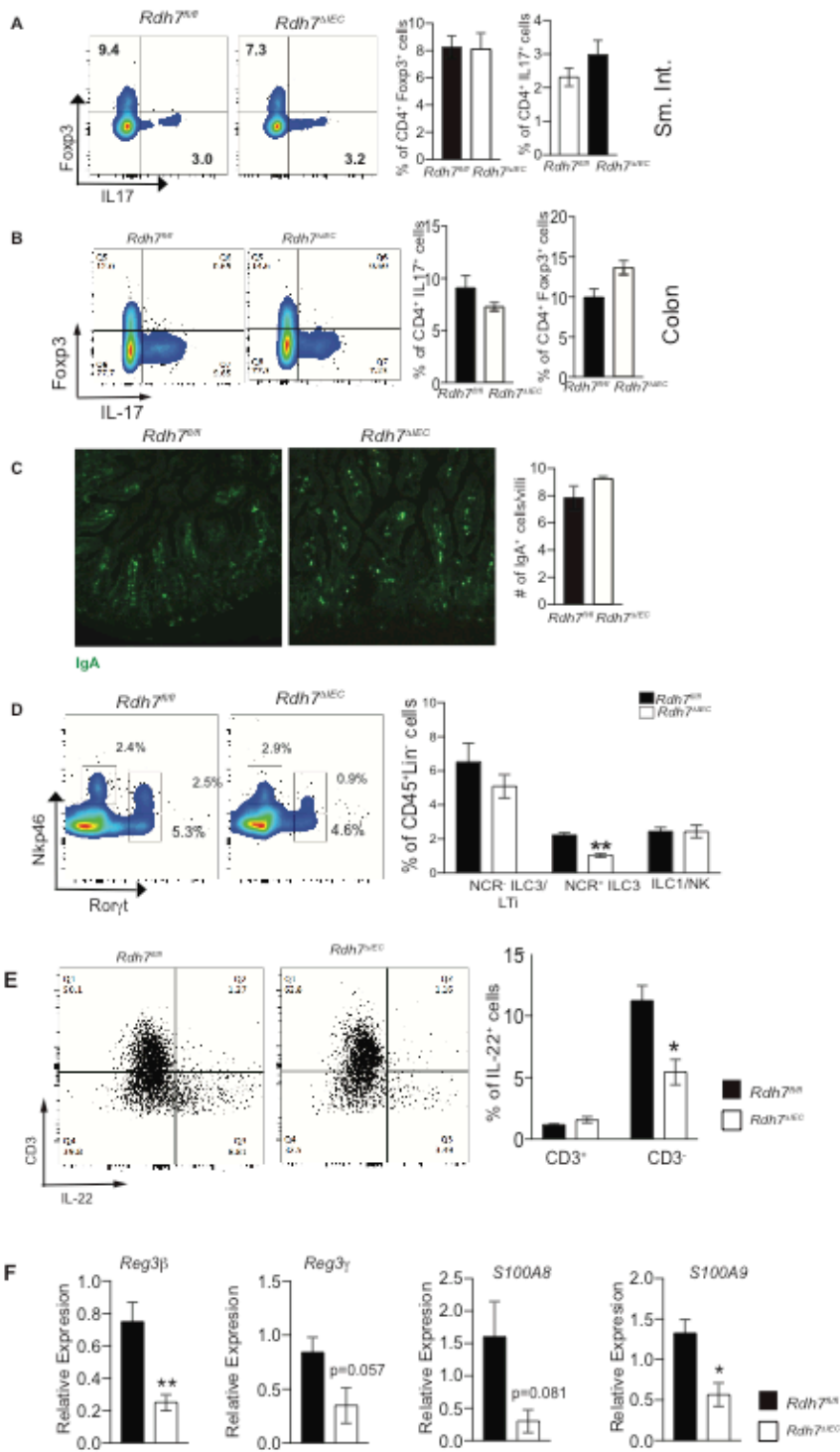


Figure S4. Analysis of gut T and B lymphocytes in *Rdh7^{fl/fl}* and *Rdh7^{ΔIEC}* mouse models. Related to Figure 5.

(A) Representative flow cytometry plots showing frequency CD45⁺CD4⁺ cells. Bar plots show frequencies of T-reg and Th17 cells in the small intestine lamina propria. (B) Representative flow cytometry plots showing frequency CD45⁺CD4⁺ cells. Bar plots show frequencies of T-reg and Th17 cells in the colon lamina propria. (C) Immunofluorescent looking at IgA positive cells (green), followed by quantification of IgA⁺ cells per villi. (D) Representative plots showing live CD45⁺Lin⁻(CD3⁻CD19⁻CD11b⁻Gr1⁻Ly76⁻) lamina propria lymphocytes and bar plot showing frequencies of Lin⁻RORγt⁺NKp46⁻ (NCR⁻ILC3/LTi), of Lin⁻RORγt⁺NKp46⁺ (NCR⁺ILC3s) and of Lin⁻RORγt⁺NKp46⁺ (ILC1/NK) lymphocytes. (E) Frequencies of CD3⁺IL-22⁺ and CD3⁻IL-22⁺ lymphocyte populations in colon of *Rdh7^{fl/fl}* and *Rdh7^{ΔIEC}* littermate mice. mRNA quantification of antimicrobials peptides in the intestine controlled by IL-22. (N=4 per group). Student's t-test; Error bars represent SEM. **P* < 0.05, ***P* < 0.01 and ****P* < 0.00

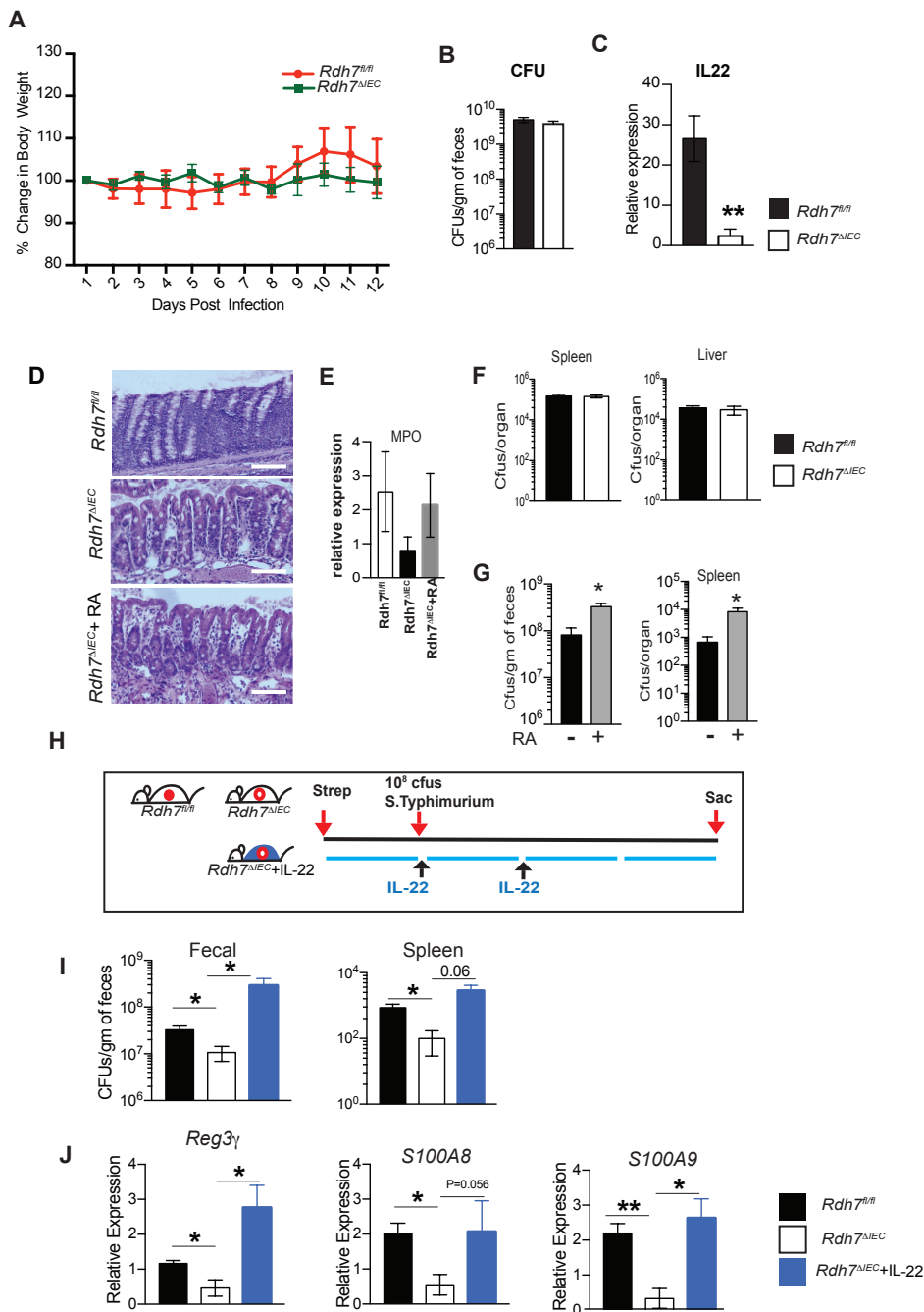


Figure S5. Role of IL-22 and RA in making *Rdh7^{fl/fl}* and *Rdh7^{ΔIEC}* mice susceptible to enteric pathogens. Related to Figure 6.

(A) Change in body weight percentage in mice that were orally given 2×10^9 CFU of *C. rodentium* and sacrificed 12 days post infection. Body weight was taken and recorded daily. (B) Colony forming units/mL (CFU/mL) were determined on the last day of the experiment. (C) Quantification of *Il-22* mRNA in colonic tissue. (D) H&E stained colon sections of *Rdh7^{fl/fl}* and *Rdh7^{ΔIEC}* and *Rdh7^{ΔIEC}+RA* during *Salmonella* infection. These images are at 10x magnification. (E) *Mpo* mRNA expression to look at neutrophil activity. (F) Quantification of bacterial burden in spleen and liver from systemic *S. Typhimurium* (Given intraperitoneally) in *Rdh7^{fl/fl}*, *Rdh7^{ΔIEC}* mice at 24 hours post infection. (G) Quantification of *S. Typhimurium* bacterial burden in feces from streptomycin treated C57 wild-type and C57 wild-type +RA mice at 72 hours post infection. (H) Diagram illustrating *S. Typhimurium* infection and IL-22 treatment timeline. (I) Quantification of *S. Typhimurium* bacterial burden in fecal content and spleen from streptomycin treated *Rdh7^{fl/fl}*, *Rdh7^{ΔIEC}* and *Rdh7^{ΔIEC}+IL-22* mice at 72 hours post infection. (J) mRNA quantification from colon tissue of IL-22 dependent antimicrobials, in the colon of *Rdh7^{fl/fl}*, *Rdh7^{ΔIEC}* and *Rdh7^{ΔIEC}+IL-22* mice 72 hours post infection with *S. Typhimurium*. Representative data of a single experiment that were performed three times (N=4 per group). Student's test; Error bars represent SEM. * $P < 0.05$, ** $P < 0.01$.

retinoid	mass	DP, V	Q1, m/z	EP, V	CE, V	Q3, m/z	XP, V
atRA	300.2	80	301.2	10	17	205.1	10
atRA-d5	305.2	80	306.2	10	17	208.1	10
ROH	286.2	60	269.2	10	25	93	10
ROH-d8	294.2	60	277.2	10	25	98	10
RE palmitate	524.5	60	269.2	10	25	93	10
RE palmitate-d4	528.5	60	273.2	10	25	94	10

Supplemental Table 1 MS/MS parameters used for retinoid quantification. Related to figure 1.

CE- collision energy, DP, declustering potential, EP entrance potential, Q1 precursor ion, Q3 product ion, XP exit potential.

Gene	Forward	Reverse
<i>Rdh7</i>	5' GTGTCTTTGTGTGGTGGTGGTTAC 3'	5' CCACAGCTTCTCTATGCTGTGTGA 3'
<i>Raldh1</i>	5' GAATTCTGCCAAGGGATTCA 3'	5' GGGGTCAGAGGATTTCCAAGAACATA 3'
<i>Raldh2</i>	5' AATCCAGCCACAGGAGAGCAAGTG 3'	5' CACGGTGTTACCACAGCACAATGC 3'
<i>Cyp26A</i>	5' GCACAAGCAGCGAAAGAAGGTGATT 3'	5' GGAAGAGAGAAGAGATTGCGGGTCA 3'
<i>Lrat</i>	5' AGTTCAAGACTAGCCTGCTCA 3'	5' TACAAGCTGGCCTTCGAC 3'
<i>Reg3β</i>	5' TACTGCCTTAGACCGTGCTTTCTG 3'	5' GACATAGGGCAACTTCACCTCACA 3'
<i>Reg3γ</i>	5' TTCCTGTCCTCCATGATCAAAA 3'	5' CATCCACCTCTGTTGGGTTCA 3'
<i>S100a8</i>	5' TGCCTCAGTTTGTGCAGAATATAAA 3'	5' TCACCATCGCAAGGAACTCC 3'
<i>S100a9</i>	5' TGTCTCAGTTTGTGCAGAATATAAA 3'	5' TCACCATCGCAAGGAACTCC 3'
<i>LacZ</i>	5' GCGTGGATGAAGACCAGC 3'	5' CGAAGCCGCCCTGTAAAC 3'
<i>Il-22</i>	5' TTGAGGTGTCCAACCTCCAGCA 3'	5' AGCCGGACGTCTGTGTTGTTA 3'
<i>Gapdh</i>	5' AACTTTGGCATTGTGGAAGG 3'	5' ACACATTGGGGGTAGGAACA 3'
<i>Isx</i>	5'TTCCACTTCACCCATTACCC 3'	5'CTCTTCTCCTGCTTCCTCCA 3'
<i>18-s</i>	5' CATTCGAACGTCTGCCCTATC 3'	5' CCTGCTGCCTTCCTTGA 3'

Supplemental Table 2 Primers Used for Quantitative PCR. Related to STAR Methods