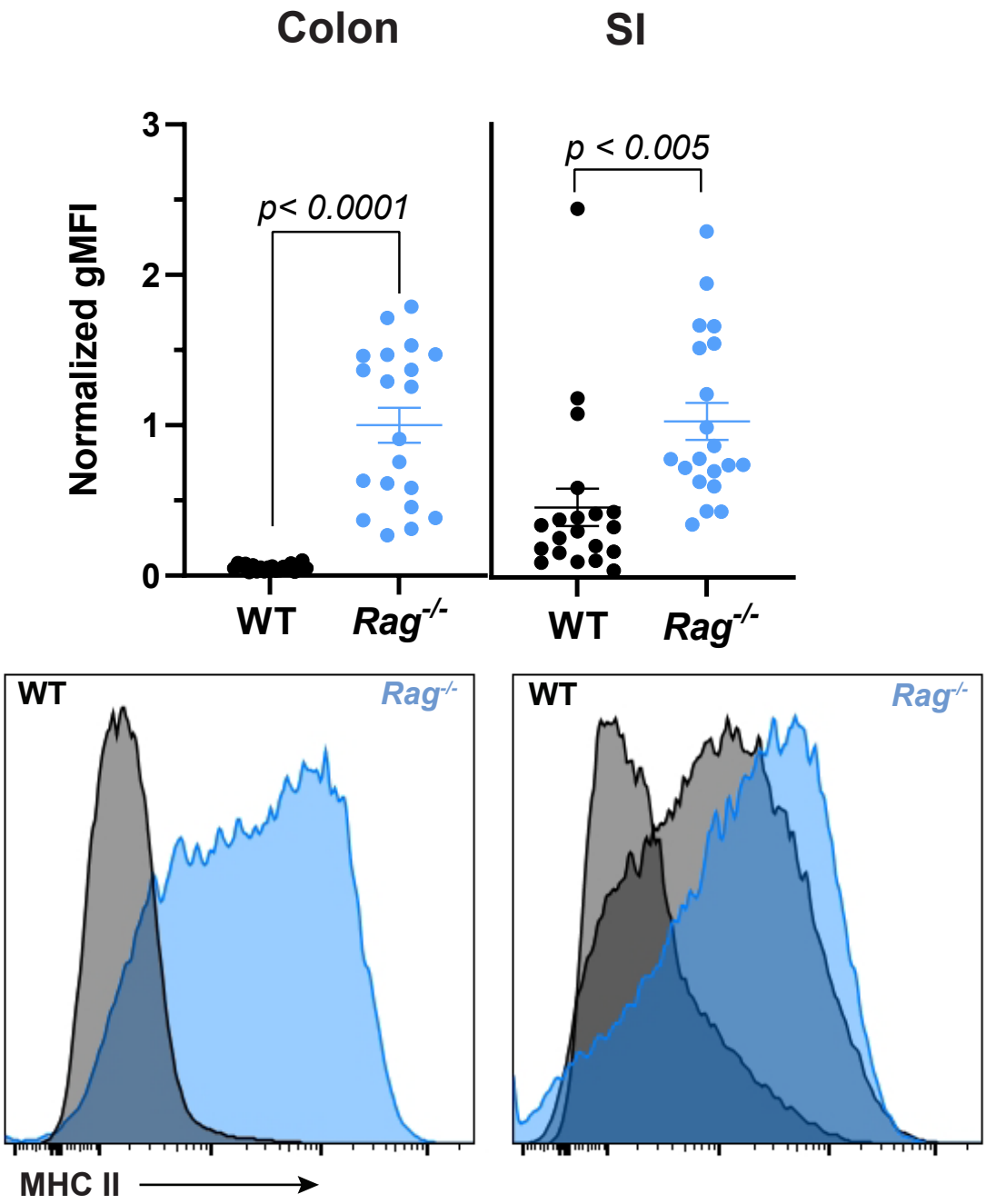
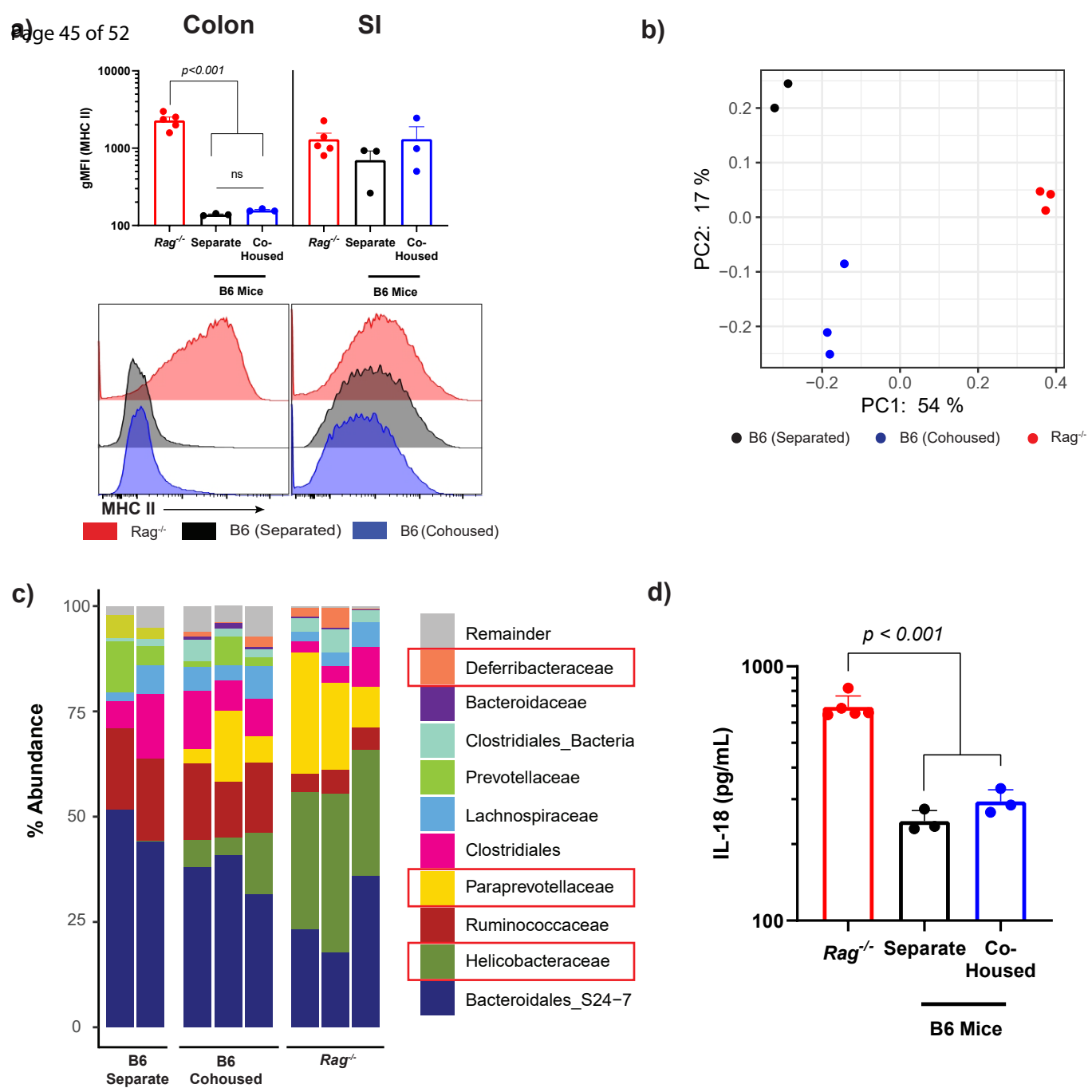
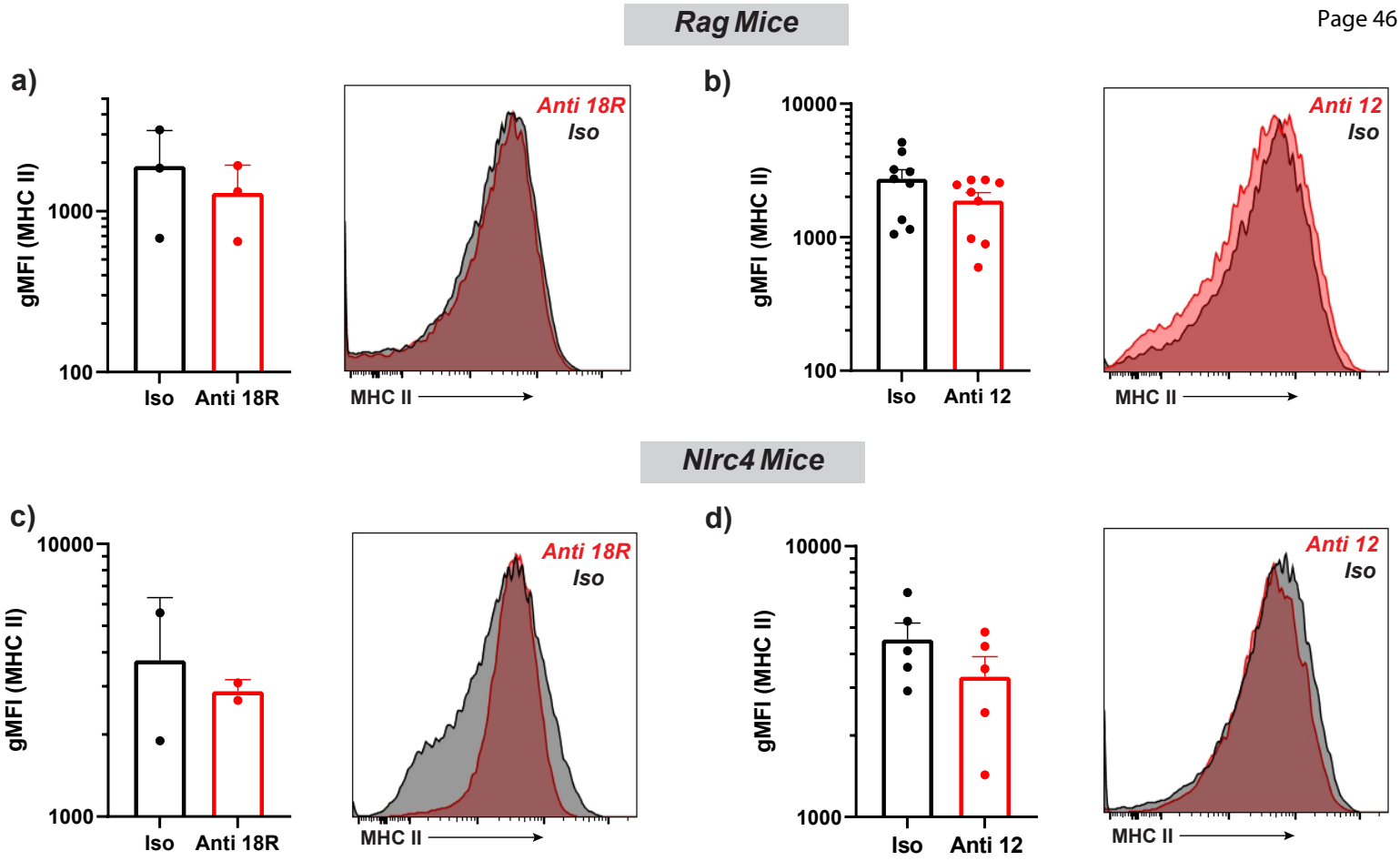


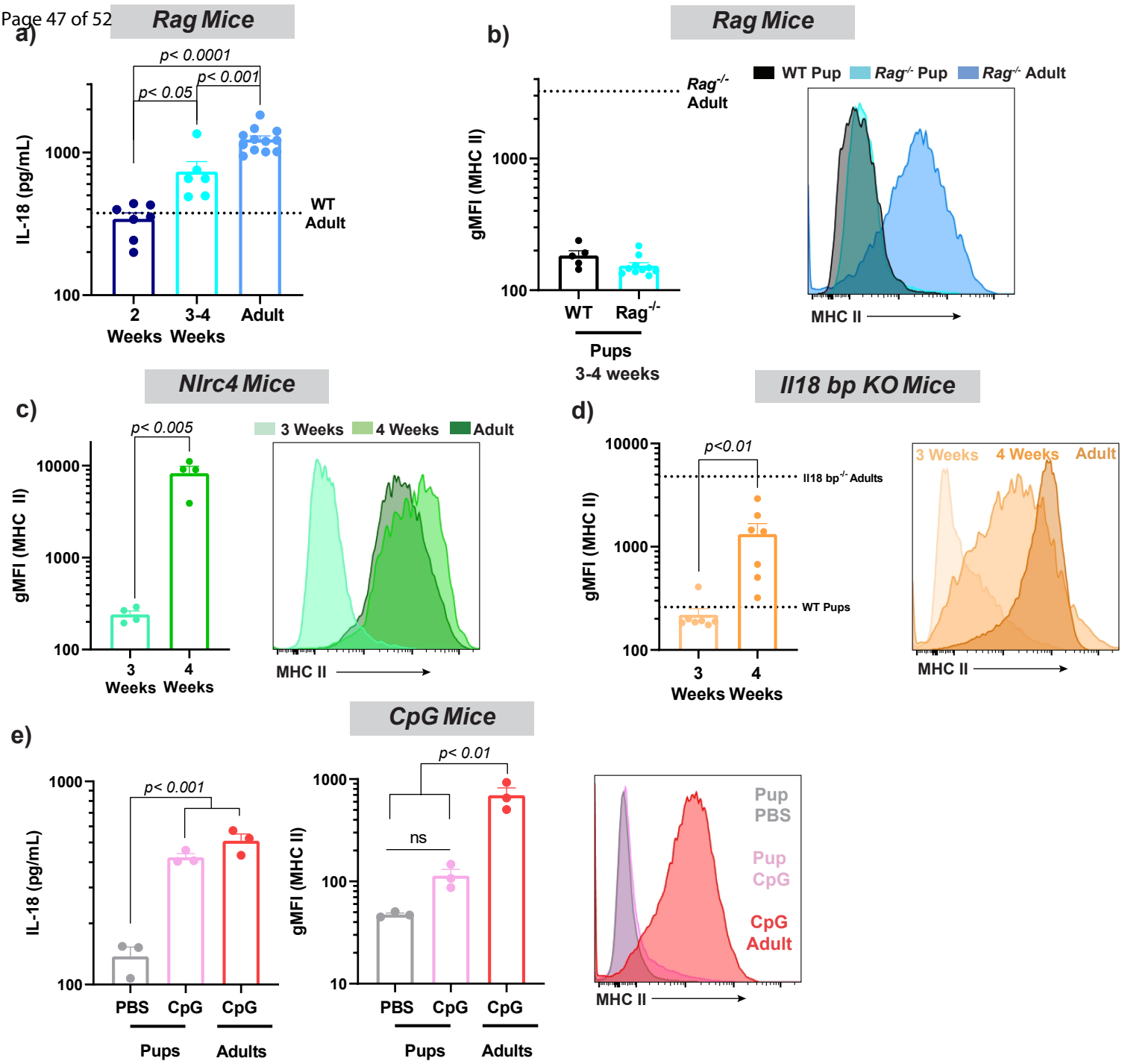
Supplemental Figure 1: Variable MHC II expression on enterocytes from WT mice. MHCII expression on Epcam^{+} colonic and small intestinal enterocytes in in house WT and *Rag1*$^{-/-}$ mice to highlight the sporadic upregulation occasionally observed in WT SIs. In house *Rag1*$^{-/-}$ consistently have high MHCII expression and serve as positive controls. gMFI was normalized to the *Rag1*$^{-/-}$ experimental average (to account for variability in MHCII staining intensity between experiments). Each mouse represents an individual data point and summarizes 5 independent experiments with 2-4 mice per genotype in each. Statistical significance is shown on the graph and was determined by unpaired t-test.



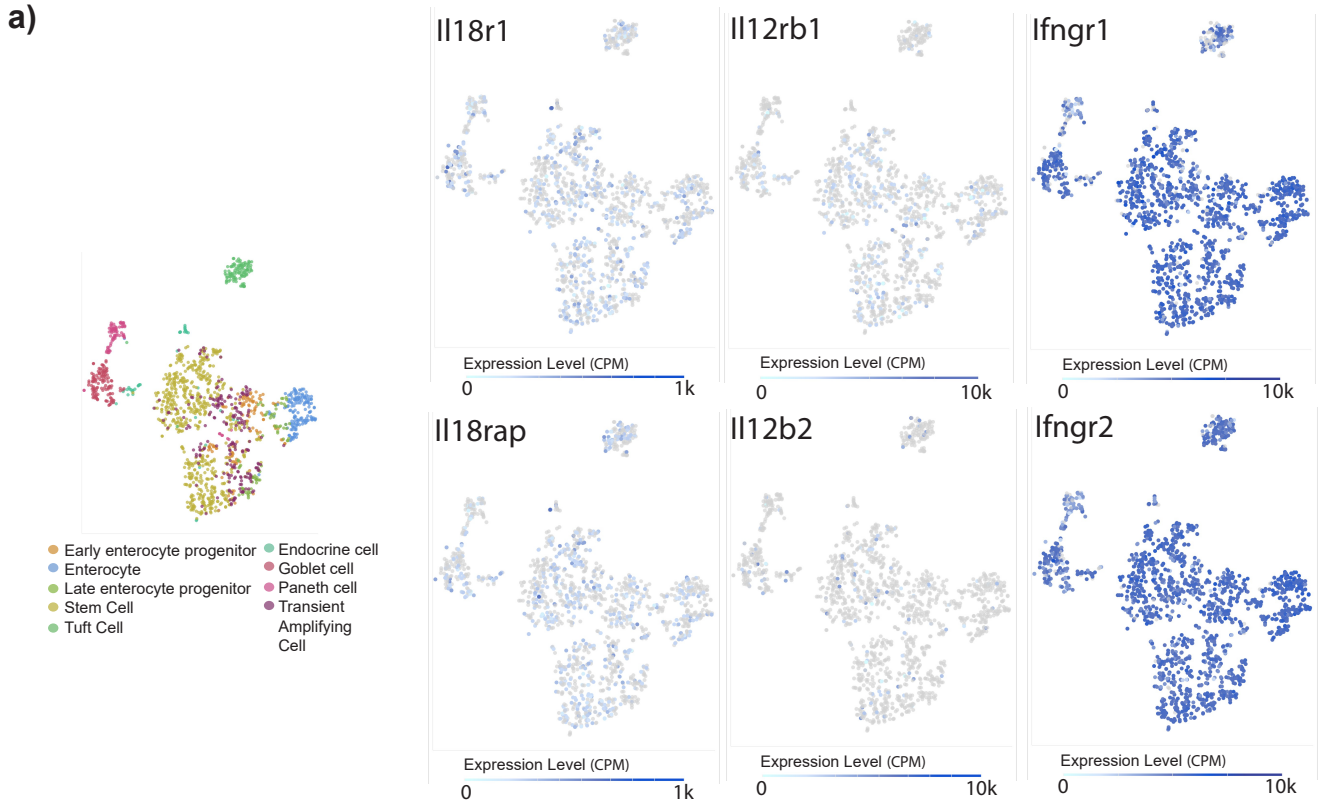
Supplemental Figure 2: Wild-type mice do not upregulate colonic IEC MHCII nor serum IL-18 upon co-housing with locally-bred *Rag1*$^{-/-}$ mice. WT (C57Bl/6J) and *Rag1*$^{-/-}$ mice were kept isolated or cohoused for 5 weeks and analyzed for epithelial MHCII expression (a). Each dot represents a single mouse and is reprentative of 2 independent experiments with 3-5 mice per housing condition. To assess alterations in microbial diversity following cohousing cecal stool was collected and genomic DNA isolated for 16S analysis. Jaccard principal coordinate analysis plots of cecal fecal diversity (b) and family diversity (c), for each mouse. Serum IL-18 at sacrifice (d). gMFI=geometric mean fluorescent intensity, PC=Principal Coordinate.

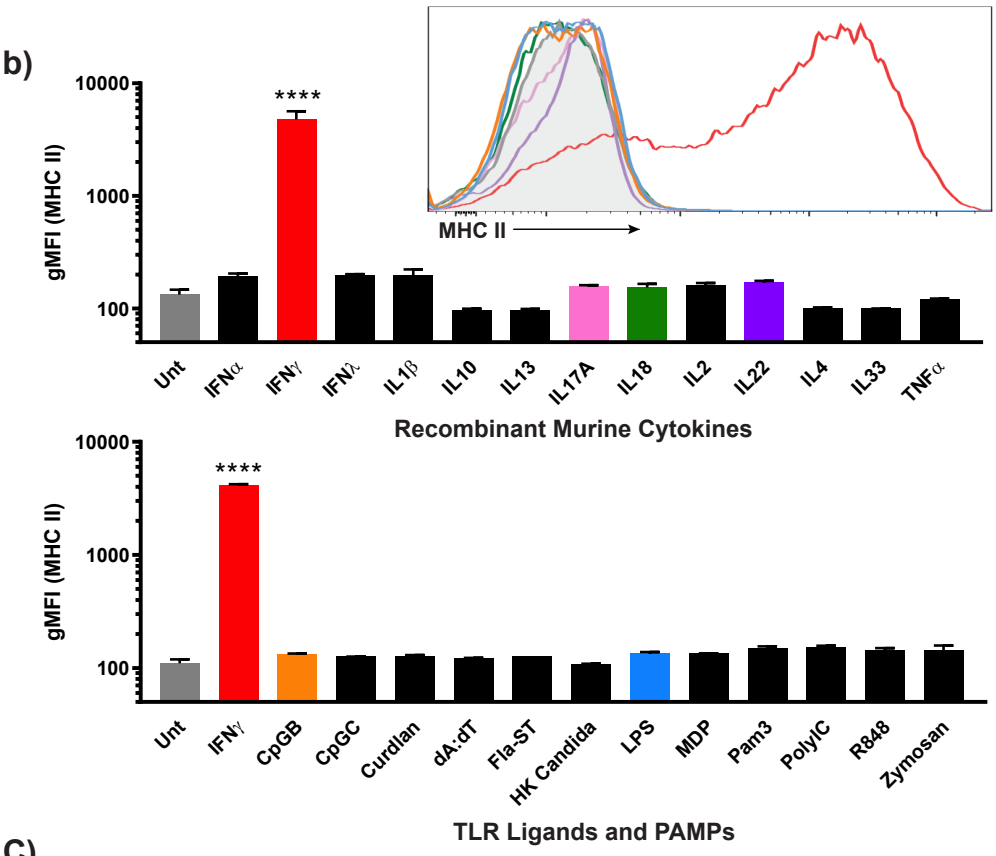


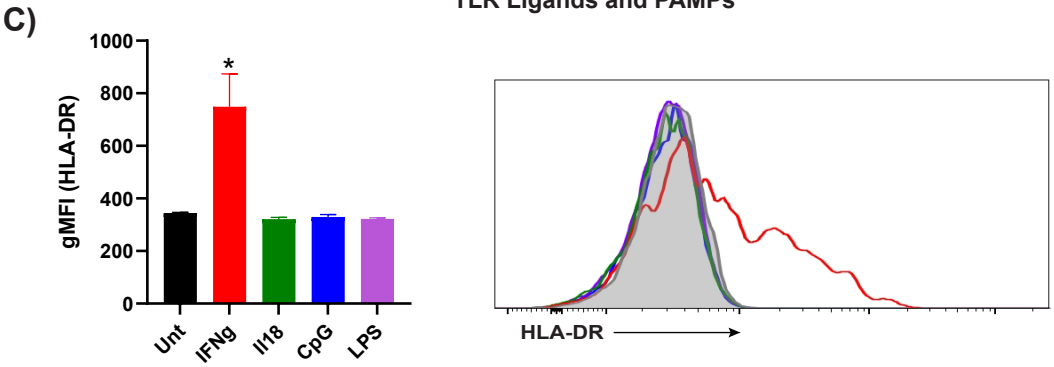
Supplemental Figure 3: Insensitivity of murine SI IEC MHCII to systemic IL-18 and IL-12 blockade. gMFI and representative histograms of MHCII expression on Epcam+ SI IECs. *Rag1*$^{-/-}$ and *Nlrc4*$^{TS/TS}$ mice were injected i.p. every three days with IL-18R (a, c) or IL-12 (b, d) blocking antibodies or isotype controls and analyzed 15 days post treatment initiation for IEC MHCII expression. Figures are representative of n=2 experiments (a, d) with 3-5 mice per treatment, the combined result of n=2 experiments with 4-5 mice per treatment or of a single experiment with n= 2 mice (c). All results were deemed non-significant by unpaired T test.



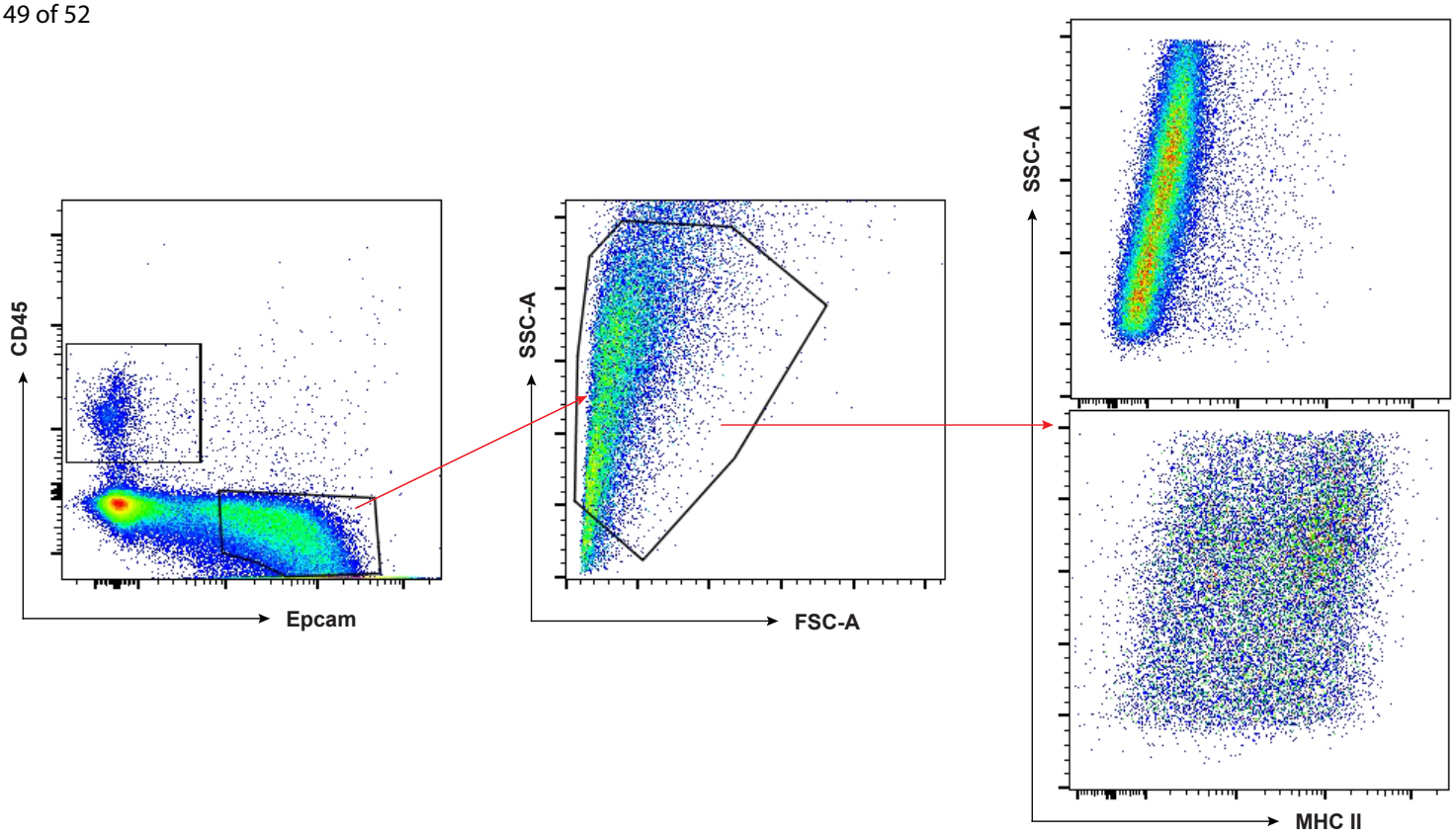
Supplemental Figure 4: MHCII induction is age dependent. Time course of small intestinal (SI) epithelial MHCII expression and serum IL-18 by age. Serum IL-18 (a) and MHCII expression (b) in 3-4 week old *Rag1*$^{-/-}$ pups vs adults. SI MHCII in *NLRC4*$^{TS/TS}$ (c) and IL18BP knockouts pups (d). Five-day old pups and adults were injected daily with CpG (ip), sacrificed 5 days post treatment initiation, and analyzed for SI MHC II and serum IL-18 (e). Normalized read counts from bulk RNA-seq of FACS-sorted IECs from neonatal intestine derived from mice four hours after subcutaneous injection of saline [PMID: 32847859]. Gapdh and Foxp3 are included as positive and negative controls, respectively. Figures b and e are representative of two independent experiments with 2-4 mice per experimental group. Figures a, c and d show combined data from two independent experiments with each mouse denoted by a single datapoint. Statistical significance is shown on each graph and was determined by unpaired t-test (n = 2 groups) or one-way ANOVA (n = 3 groups).







Supplemental Figure 5: IFNg is a unique and potent driver of IEC MHCII expression in vitro. t-SNE plot (perplexity=25) colored by inferred cell type or gene expression of select murine cytokine receptors from Haber et al [PMID: 29144463] visualized in Single Cell Expression Atlas (a). WT B6 small intestinal organoids (b) or human small intestinal enteroids (c) were treated for 3.5 days with recombinant cytokines, TLR ligands, and pathogen-derived products and epithelial MHCII/HLA-DR expression analyzed via flow cytometry. Representative histograms with select treatments are shown. The murine cytokines were run in triplicate and are representative of n = 2 experiments, while the murine TLR ligands are duplicates. The human samples were run in duplicate and are representative of n = 2 independent experiments. * $p < 0.05$ and **** $p < 0.0001$ by one-way ANOVA. Il-12rb1/2= IL-12 receptor beta 1/2, IL18R1= IL18 receptor 1, IL18Rap= IL18 receptor accessory protein, gMFI=geometric mean fluorescent intensity, IFNgR1/2= IFNg receptors 1/2, Unt=untreated.



Supplemental Figure 6: IEC identification from colonic intestinal epithelial scrapes. Gating of the colonic intestinal epithelium in Flow Jo showing MHCII high vs MHCII low mice.

Supplemental Table 1: TLR ligand and PAMP stimuli sources and concentrations used in Supplmental Figure 5.

Compound	Source	Treatment Dose
Pam3CSK4	Invivogen	1ug/ml
Zymozan	Invivogen	10ug/ml
Polyinosinic: polycytidylic acid (Poly I:C)	Sigma	100ug/ml
Lipopolysaccharide (LPS)	Sigma	1 ug/ml
Flagellin from *Salmonella typhimurium* (Fla-ST)	Invivogen	0.1ug/ml
Resiqimod (R848)	Invivogen	10ug/ml
CpG-B 1826	IDT	1ug/ml
CpG-C	Invivogen	1uM/ml
Muramyldipeptide (MDP)	Invivogen	10ug/ml
Curdlan-AL	Sigma	100ug/ml
Heat Killed Candida	Sarah Gaffen University of Pittsburgh [*]	1:100 Dilution from Stock
dA:dT	Invivogen	400ng/ml

* Hernandez-Santos, N. et al. Th17 cells confer long-term adaptive immunity to oral mucosal Candida albicans infections. Mucosal Immunol 6, 900-910 (2013).

NOTE: Please save this file locally before filling in the table, DO NOT work on the file within your internet browser as changes will not be saved. Adobe Acrobat Reader (available free here) is recommended for completion.

The ARRIVE guidelines 2.0: author checklist

The ARRIVE Essential 10

These items are the basic minimum to include in a manuscript. Without this information, readers and reviewers cannot assess the reliability of the findings.

Item		Recommendation	Section/line number, or reason for not reporting
Study design	1	For each experiment, provide brief details of study design including: a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated. b. The experimental unit (e.g. a single animal, litter, or cage of animals).	The details can be found in the materials and methods section and in the individual figure legends. The experimental unit was a single animal.
Sample size	2	a. Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used. b. Explain how the sample size was decided. Provide details of any *a priori* sample size calculation, if done.	These details are provided in the Figure legends as they were different for each experiment and experimental condition. Sample size was chosen based upon the availability of select murine genotypes and the minumum number needed to acheive statistical significance.
Inclusion and exclusion criteria	3	a. Describe any criteria used for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established *a priori.* If no criteria were set, state this explicitly. b. For each experimental group, report any animals, experimental units or data points not included in the analysis and explain why. If there were no exclusions, state so. c. For each analysis, report the exact value of *n* in each experimental group.	With the exception of an animal in which we failed to obtain viable cells from a lamina propria prep all mice were included in the experimental analysis. In Figure 8 we failed to obtain sufficent vaible cells for subsequent analyis from a single animal. This is why we state in the legend that n=1-2 animals were used in each experiment. This can be found in the Figure Legends.
Randomisation	4	a. State whether randomisation was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomisation sequence. b. Describe the strategy used to minimise potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly.	Whenever possible control and experimental groups were cohoused (blocked mice and non blocked mice in the same cage). Experimental mice were cohoused as often as possible.
Blinding	5	Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).	The primary person conducting the experiment and the person performing the subsquent flow analyis were not blinded in select experiments due to (cage labelling requirements in the animal facility prevented this). This was done to avoid confusion when mulitple genotypes were used in a single experiment. We also have very distinct
Outcome measures	6	a. Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes). b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size.	We assessed MHCII expression by flow cytometry and serum IL-18 by Cytometric Bead Array. The other important details can be found in the materials and methods. Each experiment ulilized different numbers of mice. The speific details can be found in the figure legends.
Statistical methods	7	a. Provide details of the statistical methods used for each analysis, including software used. b. Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met.	Details are provided in the figure legends, but consisted of either unpaired T tests or One way ANOVAs. The data met the assumptions
Experimental animals	8	a. Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight. b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures.	All details are provided in the methods or figure legends. Both sexes were included in the experiments. We used a variety of mice that had been genetically modified. The details can be found in the materials and methods.
Experimental procedures	9	For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including: a. What was done, how it was done and what was used. b. When and how often. c. Where (including detail of any acclimatisation periods). d. Why (provide rationale for procedures).	The details can be found in the materials and methods section and the individual figure legends. All studies were conducted at the University of Pittsburgh in SPF conditions. see above All mice from external sources underwent a 48 h acclimitization prior to the start of the experiments. see above
Results	10	For each experiment conducted, including independent replications, report: a. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range). b. If applicable, the effect size with a confidence interval.	These details can be found in the Materials and Methods section and in the figure legends. These details can be found in the Materials and Methods section and in the figure legends.

The Recommended Set

These items complement the Essential 10 and add important context to the study. Reporting the items in both sets represents best practice.

Item		Recommendation	Section/line number, or reason for not reporting
Abstract	11	Provide an accurate summary of the research objectives, animal species, strain and sex, key methods, principal findings, and study conclusions.	The objective of the study was to determine the
Background	12	a. Include sufficient scientific background to understand the rationale and context for the study, and explain the experimental approach. b. Explain how the animal species and model used address the scientific objectives and, where appropriate, the relevance to human biology.	See Introduction and Results sections Mice like humans are known to have variable MHCII expression on intestinal epithelial cells. Mice offer the additional benefit to human studies with respect to the generation of knock in, knock out or mutant mice. Many of these tools were utilized to identify the
Objectives	13	Clearly describe the research question, research objectives and, where appropriate, specific hypotheses being tested.	Our research objective was to dissect the mech
Ethical statement	14	Provide the name of the ethical review committee or equivalent that has approved the use of animals in this study, and any relevant licence or protocol numbers (if applicable). If ethical approval was not sought or granted, provide a justification.	All studies were approved by the University of Pittsburgh Animal Care and Use committee (Protocol #: 19126461)
Housing and husbandry	15	Provide details of housing and husbandry conditions, including any environmental enrichment.	Animals were housed on 12 hour light dark cyc
Animal care and monitoring	16	a. Describe any interventions or steps taken in the experimental protocols to reduce pain, suffering and distress. b. Report any expected or unexpected adverse events. c. Describe the humane endpoints established for the study, the signs that were monitored and the frequency of monitoring. If the study did not have humane endpoints, state this.	Animals were monitored a minimum of twice weekly for signs of discomfort (hunching, reduced grooming, weight loss). Our Rag KO mice reconsituted with either WT or IFNg KO bone marrow received therapeutic subcutaneous saline to combat weight loss that occured following irradiation and reconstitution. This experiment was terminated prior to the onset of adverse Mice were monitored a minimum of twice weekly for signs of discomfort (hunching, reduced grooming and weight loss. With the exception of the Rag KO mice irradiated and reconsituted with IFNg or WT bone marrow no adverese reactions were observed, nor
Interpretation/ scientific implications	17	a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature. b. Comment on the study limitations including potential sources of bias, limitations of the animal model, and imprecision associated with the results.	These details can be found in the results and discussion sections of the manuscript Bias was not noted in the experiment. There were limitations associated with determining the gamma production from the KLRG1+ NK1.1+ cells population due to low cells counts and increased mortlity of these cells following stimulation
Generalisability/ translation	18	Comment on whether, and how, the findings of this study are likely to generalise to other species or experimental conditions, including any relevance to human biology (where appropriate).	The IL-18/IL-12 -IFNg axis is likely to be similar in humans, as we show that human small intestinal organoids induce MHCII expression in response to IFNg, as well.
Protocol registration	19	Provide a statement indicating whether a protocol (including the research question, key design features, and analysis plan) was prepared before the study, and if and where this protocol was registered.	All studies were part of the current protocol provided. Previous work was taken from published data and is referenced accordingly in the text
Data access	20	Provide a statement describing if and where study data are available.	Pending publication the 16S sequencing data v
Declaration of interests	21	a. Declare any potential conflicts of interest, including financial and non-financial. If none exist, this should be stated. b. List all funding sources (including grant identifier) and the role of the funder(s) in the design, analysis and reporting of the study.	The lead authors SWC has a conflict of interest, which is described on the cover page and below: COI: SC has received research support for a SC, CS, and VD were supported by NICHD R01HD098428. LVDK and AHPB were supported by Autoimmunity and Immunopathology T32 (5T32AI089443-11). TH was supported by the Kenneth Rainin Foundation and R01DK120697. The