Vertically transmitted faecal IgA levels determine extra-chromosomal phenotypic variation

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The proliferation of genetically modified mouse models has exposed phenotypic variation between investigators and institutions that has been challenging to control1–5. In many cases, the microbiota is the presumed cause of the variation. Current solutions to account for phenotypic variability include littermate and maternal controls or defined microbial consortia in gnotobiotic mice6–9. In conventionally raised mice, the microbiome is transmitted from the dam2,8,9. Here we show that microbially driven dichotomous faecal immunoglobulin A (IgA) levels in wild-type mice within the same facility mimic the effects of chromosomal mutations. We observe in multiple facilities that vertically transmissible bacteria in IgA-low mice dominantly lower faecal IgA levels in IgA-high mice after co-housing or faecal transplantation. In response to injury, IgA-low mice show increased damage that is transferable by faecal transplantation and driven by faecal IgA differences. We find that bacteria from IgA-low mice degrade the secretory component of secretory IgA as well as IgA itself. These data indicate that phenotypic comparisons between mice must take into account the non-chromosomal hereditary variation between different breeders. We propose faecal IgA as one marker of microbial variability and conclude that co-housing and/or faecal transplantation enables analysis of progeny from different dams.

We chose to study the role of secretory immunoglobulin A (SIgA), which is a critical intersection between the host immune system and the microbiota10. While interrogating baseline intestinal IgA levels in wild-type (WT) C57BL/6J (B6) mice, we observed a binary phenotype in faecal IgA levels between cages (Fig. 1a): those with high faecal IgA (defined as 0.05–0.25 μg IgA per milligram of faeces) and those with nearly undetectable faecal IgA, hereafter designated as IgA-high and IgA-low mice, respectively. We observed this differential IgA phenotype in two separate facilities at our institution in independently derived WT B6 colonies (Extended Data Fig. 1a). Although both facilities are specific pathogen-free, the protocols, access, and personnel are distinct. All experiments were performed in both facilities unless otherwise noted. Despite the profound difference in faecal IgA, serum IgA levels were similar between these two groups, suggesting a gut-specific effect (Fig. 1b). The binary phenotype was passed from breeders to progeny, indicating a vertically transmissible phenotype (Fig. 1c). Furthermore, this phenotype was laterally transferable by co-housing IgA-high and IgA-low mice. Remarkably, both IgA-high and IgA-low mice were found to be IgA-low after co-housing (Fig. 1d). This result also occurred by cross-transfer experiments involving faecal transplantation between mice in our two facilities (Extended Data Fig. 1b, c). Hence, the IgA-low phenotype was dominant, indicating that faecal IgA levels can be regulated by suppression and not only induction.

We next passaged microbes through polymeric Ig receptor mutant (pIgR−/−) mice that lacked the ability to transport IgA into the lumen11. This experiment allowed us to test whether the stability of the faecal microbiome creating this binary phenotype requires the presence of faecal SIgA (Extended Data Fig. 2a). Faecal samples from pIgR−/− mice transplanted with IgA-low material conferred the IgA-low phenotype to IgA-high WT mice (Extended Data Fig. 2b). Thus, exposure to a novel environment lacking SIgA (the pIgR−/− intestine) did not affect the ability of the faecal microbiota to regulate the IgA-high versus IgA-low phenotype.

Because commensal bacteria and viruses modulate mucosal IgA12,13, we transplanted IgA-high mice with IgA-low faecal material filtered to remove large microbes (for example, bacteria, fungi). Mice transplanted with filtrate remained IgA-high, while mice transplanted with unfiltered material became IgA-low (Fig. 1e), implicating intestinal microbes and excluding filterable viruses. To determine whether specific microbial pools could induce the IgA-low phenotype, we pre-treated IgA-low mice with a broad-spectrum antibiotic cocktail (vancomycin, neomycin, ampicillin, and metronidazole; VNAM), then performed faecal transplant from IgA-high or IgA-low mice (Extended Data Fig. 2c). Transplantation with IgA-high microbes increased faecal IgA, indicating that VNAM eliminated IgA-low-associated microbes (Fig. 1f). We found that ampicillin but not metronidazole was sufficient to reverse the IgA-low phenotype, indicating ampicillin-sensitive microbe(s) were responsible for the IgA-low phenotype (Fig. 1g and Extended Data Fig. 2d). Unlike VNAM, ampicillin treatment reversed the IgA-low phenotype without transplantation, suggesting VNAM eliminated both IgA-suppressive and IgA-inductive microbes while ampicillin eliminated only IgA-suppressive microbes (Fig. 1f, g). We assessed whether the faecal IgA status of treated mice was vertically transmissible, and found that VNAM-treated IgA-low mice transplanted with IgA-high samples gave rise to IgA-high progeny (Extended Data Fig. 2e). Taken together, these results support a model where the IgA-low phenotype is bacterially driven, transmissible, and dominant.

Previous studies have shown that pIgR−/− mice are more susceptible to dextran sodium sulphate (DSS) injury14,15. With DSS treatment, IgA-low mice lost significantly more weight than their IgA-high counterparts (Fig. 2a, 2c). This DSS sensitivity could be secondary to diminished SIgA or altered microbial composition. To address these possibilities, we re-colonized WT and pIgR−/− mice with IgA-high or IgA-low faecal material after VNAM treatment (Extended Data Fig. 2a) and before DSS treatment (Extended Data Fig. 3a). As expected, WT + IgA-high mice showed enhanced DSS sensitivity compared with WT + IgA-high mice (Fig. 2d, e and Extended Data Fig. 3b). We observed increased weight loss and colonic ulceration in pIgR−/− mice compared with WT mice, a finding consistent with previous reports14,15. Interestingly, this sensitivity was independent of IgA-low microbes, as pIgR−/− mice colonized with IgA-high or IgA-low microbes had no significant differences in weight loss or ulceration (Fig. 2e, d and Extended Data Fig. 3b). This finding implied that altered SIgA levels, and not the microbes themselves, caused increased DSS damage in WT + IgA-low mice.

To study the mechanism by which IgA-low microbes suppress faecal IgA, we assessed IgA production and transport capacity by pIgR in...
IgA-high versus IgA-low mice. We found no difference in lamina propria plasma cell numbers between these mice (Extended Data Fig. 4a–f), nor in immunofluorescence staining for plgR (Extended Data Fig. 4g–j).

On the apical surface of intestinal epithelial cells, plgR is cleaved and the extracellular portion is released into the lumen bound to its ligand (multimeric immunoglobulins containing the J chain, such as dimeric IgA)16. This cleaved form of plgR, called bound secretory component (secretory component degradation in IgA-low mice, we performed 16S rDNA sequencing of IgA-high and IgA-low faecal samples. Comparison of samples within individual facilities revealed taxonomic biomarkers associated with faecal IgA levels (Extended Data Fig. 5a, b and c). The only genus-level IgA-low biomarker compared in IgA-high and IgA-low mice after VNAM treatment + IgA-high/IgA-low faecal transplant: percentage initial weight; percentage of ulcerated distal colon. Two-way repeated measures ANOVA: a, column factor $P < 0.0001$; b, $F = 15.56, P < 0.0001$. c, Faecal IgA from IgA-high mice pre- (n = 18) and post-faecal transplant (FT) with unfiltered (post-FT, n = 13) or 0.45-μm-filtered faecal material (post-filter FT, n = 5) from IgA-low mice. f, Faecal IgA of post-FT mice pre-treated with (f) VNAM or (g) ampicillin (Amp). One-way ANOVA: e, $F = 5.685, P = 0.0076$; f, $F = 16.15, P < 0.0001$, n = 16 (pre-VNAM), n = 18 (post-VNAM), n = 9 (post-IgA-high FT), n = 8 (post-IgA-low FT); g, $F = 22.96, P < 0.0001$, n = 22 (pre-Amp), n = 28 (post-Amp), n = 12 (post-IgA-high FT), n = 12 (post-IgA-low FT). All values are mean ± s.e.m. Different footnote symbols indicate groups significantly different by Tukey’s multiple comparison test (P < 0.05). Dashed lines, limit of detection.

(Amazon) IgA-high mice had reduced faecal secretory component (Fig. 3a–c), but IgA-low mice had increased faecal proteases17,18. We hypothesized that low SlgA levels were secondary to secretory component degradation. By immunoblotting for plgR/secretory component in faecal samples and whole tissue, we found that IgA-high and IgA-low mice had comparable tissue plgR levels (Fig. 3a–c), but IgA-low mice had increased faecal secretory component (Fig. 3a–c). To identify potential microbe(s) responsible for enhanced secretory component degradation in IgA-low mice, we performed 16S rDNA sequencing of IgA-high and IgA-low faecal samples. Comparison of samples within individual facilities revealed taxonomic biomarkers associated with faecal IgA levels (Extended Data Fig. 5a, b and c).

### Figure 1

Low faecal IgA in WT mice is a vertically and horizontally transferable, dominant phenotype driven by ampicillin-sensitive bacteria.

- **a.** Faecal IgA (a) and serum IgA (b) by enzyme-linked immunosorbent assay (ELISA). Mann–Whitney U-test: $P < 0.0001$, n = 40 (IgA-high), n = 34 (IgA-low) mice; b, $P = 0.4704, n = 12$ per group. c, Faecal IgA from IgA-high (n = 10) and IgA-low (n = 11) breeders and their adult progeny (IgA-high (n = 12), IgA-low (n = 9)), IgA-high (n = 11) and after co-housing (IgA-high (n = 8), IgA-low (n = 10)). One-way analysis of variance (ANOVA): $C, F = 45.95, P < 0.0001$; d, $F = 15.56, P < 0.0001$. e, Faecal IgA from IgA-high mice pre- (n = 18) and post-faecal transplant (FT) with unfiltered (post-FT, n = 13) or 0.45-μm-filtered faecal material (post-filter FT, n = 5) from IgA-low mice. f, Faecal IgA of post-FT mice pre-treated with (f) VNAM or (g) ampicillin (Amp). One-way ANOVA: e, $F = 5.685, P = 0.0076$; f, $F = 16.15, P < 0.0001$, n = 16 (pre-VNAM), n = 18 (post-VNAM), n = 9 (post-IgA-high FT), n = 8 (post-IgA-low FT); g, $F = 22.96, P < 0.0001$, n = 22 (pre-Amp), n = 28 (post-Amp), n = 12 (post-IgA-high FT), n = 12 (post-IgA-low FT). All values are mean ± s.e.m. Different footnote symbols indicate groups significantly different by Tukey’s multiple comparison test (P < 0.05). Dashed lines, limit of detection.

### Figure 2

The IgA-low phenotype alters susceptibility to DSS in an IgA-dependent manner. a–c, DSS treatment of IgA-high/IgA-low mice: a, percentage initial weight; b, percentage of ulcerated distal colon; c, representative haematoxylin and eosin-stained histological sections of IgA-high (n = 10) and IgA-low (n = 11) mice. d, E. coli treatment of WT and IgA-high/IgA-low mice after VNAM treatment + IgA-high/IgA-low faecal transplant: percentage initial weight; percentage of ulcerated distal colon. Two-way repeated measures ANOVA: a, column factor $P < 0.0001$, Sidak’s multiple comparisons test, final time point, IgA-high (n = 10) and IgA-low (n = 11) mice; d, column factor $P < 0.0001$, n = 15 (WT + IgA-high), n = 18 (WT + IgA-low), n = 20 (IgA-high + IgA-high), n = 21 (plgR^- + IgA-high); Tukey’s multiple comparison test, final time point. Unpaired t-test: b, $P = 0.0385$, IgA-high (n = 10), IgA-low (n = 11) mice. One-way ANOVA: e, $F = 8.272, P = 0.0007$, n = 3 (WT + IgA-high), n = 6 (WT + IgA-low), n = 8 (plgR^- + IgA-high), n = 10 (plgR^- + IgA-low). All values are mean ± s.e.m. Different footnote symbols indicate groups significantly different by Tukey’s multiple comparison test (P < 0.05). Scale bars, 1 mm. Boxes, ulcerated areas.
causative IgA-low microbes were culturable in these conditions (Fig. 3f, g). From these results, we concluded the IgA-low-inducing microbes might include Sutterella species.

We next employed cultured microbes to explore the mechanism of secretory component degradation. We generated polarized, differentiated monolayers of primary intestinal epithelial cells in Transwells and assessed dimeric IgA transport from the basolateral to the apical compartment. Treatment with γ-secretase inhibitor DAPT (N-(3,5-difluorophenacetyl)-l-alanyl)-S-phenylglycine t-butyl ester) and lipopolysaccharide (LPS) robustly induced pIgR in these cells (Extended Data Fig. 7a), which is necessary for IgA transport across the epithelium. After addition of IgA to the basal compartment, we detected secretory component in the apical supernatant of DAPT + LPS-treated cells (Fig. 4a–c). We co-cultured monolayers with either pelleted bacteria or

**Figure 3** The IgA-low phenotype correlates with an absence of faecal secretory component and the presence of specific microbial taxa *in vitro*. a. Representative anti-pIgR/secretory component (SC) immunoblots of intestinal tissue/faecal samples from IgA-high/IgA-low mice. b–d. Quantification of immunoblots for (b) ileal, (c) colonic, and (d) faecal pIgR/secretory component. Mann–Whitney U-test: b, $P = 0.7424$, $n = 7$ (IgA-high), $n = 5$ (IgA-low) mice; c, $P = 0.1285$, $n = 10$ (IgA-high), $n = 15$ (IgA-low) mice; d, $P = 0.0025$, $n = 7$ (IgA-high), $n = 5$ (IgA-low) mice. e. Relative abundance of order/family/genus 16S rDNA sequence assignments of culture inoculate from IgA-high/IgA-low samples (Pre-) or overnight cultures of these inoculates (Post-), $n = 4$ samples per group. Statistical analysis: Extended Data Table 2. f. Schematic of culturing/administration experiments. g. Faecal IgA measured in IgA-high mice pre- ($n = 18$) and post-cultured mouse administration (+IgA-high culture ($n = 10$), +IgA-low culture ($n = 9$)) depicted in f. One-way ANOVA: $F = 18.60$, $P < 0.0001$. All values are mean ± s.e.m. Different footnote symbols indicate groups significantly different by Tukey’s multiple comparison test ($P < 0.05$). Dashed lines, limit of detection.

**Figure 4** Culturable anaerobic bacteria present in IgA-low samples degrade secretory component. a–c. IgA transcytosis assay with primary intestinal epithelial Transwell monolayers apically treated with pelleted or supernatant (sup.) fraction of IgA-high/IgA-low cultures. Secretory component in apical supernatants was measured by anti-secretory-component immunoblots. Representative anti-secretory-component immunoblot (a) and quantification of undegraded secretory component (red brackets) at 3 h (b) and 6 h (c). d–f, Transwell monolayers treated as in a–c, with protease inhibitor (PI) cocktail added to IgA-high/IgA-low cultures (pelleted bacterial fraction). Representative anti-secretory-component immunoblot (d) and quantification of undegraded secretory component at 3 h (e) and 6 h (f). One-way ANOVA: b, $F = 11.11$, $P < 0.0001$, $n = 5$ experiments, three containing supernatant samples; c, $F = 54.83$, $P < 0.0001$, $n = 5$; e, $F = 3.830$, $P = 0.0263$, $n = 3$; f, $F = 12.07$, $P = 0.0002$, $n = 3$. All values are mean ± s.e.m. Different footnote symbols indicate groups significantly different by Tukey’s multiple comparison test ($P < 0.05$).
culture supernatants from IgA-high or IgA-low cultures in the apical compartment (Fig. 4a–c). In co-cultures with IgA-high pelleted bacteria, we detected secretory component in apical supernatants at three and six hours with evidence of limited degradation, while co-cultures with IgA-low pelleted bacteria exhibited substantially greater secretory component degradation at 6 h. This secretory component degradation did not depend on the presence of IgA as free secretory component was also degraded by IgA-low microbes (Extended Data Fig. 7b–d). Incubation of monolayers with IgA-high/IgA-low culture supernatants did not cause secretory component degradation (Fig. 4a–c). Differences in secretory component levels were not due to differential epithelial cell pIgR expression (Extended Data Fig. 7a). Freeze/thaw of IgA-low cultures also led to secretory component degradation, indicating that live bacteria were not necessary (Extended Data Fig. 7e–g). Taken together, these data suggest bacteria in the IgA-low microbiota degrade free and bound secretory component in vitro (Fig. 4a–c), consistent with our observation of absent faecal secretory component in vivo (Fig. 3a, d). Addition of a broad-spectrum protease inhibitor cocktail partially prevented secretory component degradation, implicating proteases in this process (Fig. 4d–f). These findings are consistent with a model in which degradation of bound secretory component of SIgA by IgA-low microbes makes the IgA portion more susceptible to proteolysis. Bacteria make proteases that can cleave human IgA1/IgA2 and secretory component, although to our knowledge this has not been addressed in murine models. In addition to secretory component degradation, we found that faecal IgA was degraded and thus decreased after co-culture with IgA-low microbes, consistent with our initial in vivo observations (Extended Data Fig. 8a, b). In the future it will be of interest to identify the protease(s) involved in the degradation of secretory component and/or IgA, and to look for additional host substrates of these proteases.

This study shows that phenotypic effects can be vertically transmitted through the microbiome, which can mimic alterations of host genes. To distinguish host genetic from extra-chromosomal effects, ideally mice must be bred so that comparisons can be performed between WT and mutated mice that have equivalent microbial exposure. Hence, breeding mice that are heterozygous for a given mutation is critical, even for the study of extra-intestinal phenotypes. Secondary options are faecal transplantation and co-housing. These can serve as methodological controls for phenotypic variation dependent on extra-chromosomal factors that may be easily transmissible between hosts. Lastly, faecal IgA serves as a readily measurable marker that can be compared within and between facilities or institutions to compare phenotypic differences.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Author Information 16S rDNA sequencing data have been deposited in the European Nucleotide Archive under accession number PRJEB7854. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to H.W.V. (virgin@wustl.edu) or T.S.S. (stappenb@pathology.wustl.edu).
METHODS

Mice. Animal protocols were approved by the Washington University Animal Studies Committee. All mice were maintained in one of two specific pathogen-free barrier facilities, with different procedures for maintaining food, water, and caging. In facility 2 (specialized research facility), complete cages (including food, bedding, isolator top, wires, and cage) were autoclaved after assembly, water was autoclaved and kept sterile, and a higher concentration of disinfectant was used (1:5:1 Clidox, Pharmacal Research Laboratories). Facility 1 (clinical sciences research building facility) also used autoclaved cage components but cages were assembled after autoclaving. The food was irradiated but not autoclaved, and a lower concentration of disinfectant was used (1:18:1 Clidox).

For facility 2, C57BL/6 WT mice were originally obtained from Jackson Laboratories (stock number 000664) and maintained as a breeding colony. For facility 1, multiple sources of C57BL/6 WT mice were used to create breeding colonies. Within our WT mouse colonies, multiple IgA-high and IgA-low breeders were identified and maintained as independent lines. The pIgR knockout mice (B6.129P2-Pigtrtm1Ppr/J;Mmimb) were initially obtained from the Mutant Mouse Regional Resource Center and were backcrossed to 98.4% C57BL/6. Male and female mice between 2 and 6 months of age were used, and faecal samples were only collected after mice were at least 8 weeks of age. Sample sizes used for studies reflect the number of mice needed for three independent experiments with at least two mice used per sample in each experiment, and 2–5 individuals per sample that were unique biological replicates. For animal studies, mice were confirmed to be IgA-high or IgA-low before subsequent experimental manipulation.

Mouse treatments. For co-housing studies, mice were co-housed 1:1 for 14 days. For faecal transplantation, faecal samples were collected from mice and re-suspended in sterile PBS to a final concentration of 200 mg ml^-1 by weight. Mice were orally administered 25 μl of the faecal mixture on two consecutive days.

Antibiotic treatments included 0.5 mg ml^-1 vancomycin, 1 mg ml^-1 neomycin, 1 mg ml^-1 ampicillin, and 1 mg ml^-1 metronidazole (Sigma). VNM indicates the cocktail for these combined antibiotics. For DSS experiments, 2.5% DSS (Tdb Consultancy) was administered in drinking water for 11 days. Mice were weighed daily and killed at 70% of initial body weight if needed. Intestines were taken for histology. All mice used in DSS experiments were from facility 2.

Preparation of faecal samples for ELISA and immunoblotting. Faecal samples were collected from mice and re-suspended in sterile PBS to a final concentration of 100 mg ml^-1 by weight. Supernatants were collected and stored at −20 °C until needed.

Preparation of faecal samples for bacterial culture. Faecal samples were collected from mice and re-suspended in sterile PBS to a final concentration of 200 mg ml^-1 by weight. Of this mixture, 250 μl was used to inoculate anaerobic chopped meat broth in Hungate tubes (Fisher Scientific) for overnight culturing in a 37 °C shaker incubator. Individual culture samples reflect unique biological replicates. For culture administration to mice, 25 μl were orally administered for two consecutive days. The faecal suspensions as well as the overnight cultures were used in epithelial co-culture experiments described below.

16S rDNA Illumina sequencing and analysis. Faecal pellets (individual biological replicates) were collected into 2-ml tubes (Sarstedt) with 1 mM diameter pellets were then cleaned with the 96-well format DNeasy Blood & Tissue Kit (Qiagen) according to the manufacturer’s instructions, and purified DNA samples were re-suspended at 25 ng μl^-1. For DNA purification from bacterial culture inoculate and anaerobic bacterial cultures (all individual biological replicates), the qIAmp DNA mini kit (Qiagen) was used according to the manufacturer’s protocol. Primer selection and polymerase chain reaction (PCR) were performed similarly as described previously. Briefly, each sample was amplified in triplicate, combined, and confirmed by gel electrophoresis, with Golya-barcoded primers specific for the V4 region (F515/S806). PCR protocols contained 18.8 μl RNase/DNase-free water, 2.5 μl 10 × High Fidelity PCR Buffer (Invitrogen), 0.5 μl 10 μM dNTPs, 1 μl 50 μM MgSO₄, 0.5 μl each of the forward and reverse primers (10 μM final concentration), 0.1 μl Platinum High Fidelity Taq (Invitrogen), and 1.0 μl genomic DNA. Reactions were held at 94 °C for 2 min to denature the DNA, with amplification proceeding for 26 cycles at 94 °C for 15 s, 55 °C for 30 s, and 68 °C for 30 s; a final extension of 2 min at 68 °C was added to ensure complete amplification. Amplicons were pooled and purified with 0.6× Agencourt Ampure XP beads (Beckman-Coulter) according to the manufacturer’s instructions. The final pooled samples, along with aliquots of the three sequencing primers, were sent to the Center for Genome Sciences (Washington University School of Medicine) for sequencing by the 2 × 250-base pair protocol with the Illumina MiSeq platform.

We analysed 16S sequences with Quantitative Insights Into Microbial Ecology (QIIME, version 1.8.0). Raw sequence fastq files were quality filtered and demultiplexed with the following criteria: the maximum number of consecutive low-quality bases was allowed to be 10 per sequencing read, and a lower quality threshold based on the Phred quality score was set to 19, and reverse-complement mapping barcodes were used. Close-reference operational taxonomic units sharing 97% identity were clustered with the UCLUST algorithm and assigned taxonomy according to the Greengenes database (version 13.8). Faecal samples were rarefied to 1,000 sequences for subsequent analyses and culture/culture inoculate samples were rarefied to 5,000 sequences. For culture/culture inoculate samples, a minimum relative abundance of 0.005% in at least one sample was set to filter out very rare operational taxonomic units before subsequent analysis.

Relative operational taxonomic unit abundance data were input into LEfSe to determine biomarkers with significant linear discriminant analysis effect size. Biomarkers for facility 1 and for facility 2 were identified by comparison of samples within each facility. Biomarkers for facilities 1 and 2 were identified by comparison of samples from both facilities. After Kruskal–Wallis analysis with an x value of 0.05 of all features, a linear discriminant analysis model was used to rank discriminant features by the effect size with which they differentiated classes. The threshold for logarithmic linear discriminant analysis score for discriminative features was set at 2.0. Biomarkers were graphically annotated on a taxonomic tree with GraPhlAn (publicly available at http://huttenhower.sph.harvard.edu/graphlan).

Primary intestinal epithelial cell culture. Primary colonic epithelial stem cells were isolated, grown, and maintained as three-dimensional spheroid cultures in Matrigel (BD Biosciences) as described previously. Cells were kept in 50% L-WRN conditioned media. Media were changed every 2 days, and cells were passaged every 3 days (1:3 split). Primary intestinal epithelial cell monolayers were formed as described previously. Briefly, spheroids were recovered from 3-day-old three-dimensional Matrigel cultures, trypsinized, dissociated to single cells by vigorous pipetting, and re-suspended in 50% L-WRN conditioned media containing 10 μM Y-27632 (R&D Systems). These cells were plated in Transwell inserts (Corning Costar) coated with 1 mg ml^-1 gelatin. Each individual experiment using the colonic epithelial stem cells reflects unique biological replicates.

Cell treatments. On day one (24 h after seeding the Transwells) the 50% L-WRN conditioned media supplemented with Y-27632 was removed and replaced with 0% conditioned media (Advanced DMEM/F12 containing 20% fetal bovine serum (FBS), 100 units of penicillin, 0.1 mg ml^-1 streptomycin, and 2 mM t-glutamine). At this time, any additional treatments were also administered to the cells: 1 μg ml^-1 LPS (Sigma) and 10 μM DAPT γ-secretase inhibitor (Millipore). Cells were given fresh media with the respective treatments on day two, and were treated for a total of 48 h before being used for transcytosis of dimeric IgA on day three.

IgA transcytosis assay. On day three, the Transwells were removed from the various treatment conditions, and switched to base media (Advanced DMEM/F12 supplemented with 2 mM t-glutamine only; no FBS, no antibiotics). Six hundred microlitres of base media containing 3 μg of mouse IgA (BD Pharmingen) was added to the lower compartment (final concentration of IgA = 5 μg ml^-1). In some experiments, 600 μl of base media alone (no IgA) was added to the lower compartment. Base media (100 μl, with or without various treatments) was added to the upper compartment. Treatments included 1/10 faecal bacterial suspensions (bacterial pellet or supernatant fractions), 1/10 overnight anaerobic chopped meat cultures (live or freeze/thawed), and 1/25 0.01% Triton-X100 for 30 min, and incubated with primary antibody at 4 °C overnight. Primary antibodies included goat anti-mouse pIgR (1:100, R&D Systems, catalogue number AF2800) and goat anti-mouse IgA (Alexa Fluor 488/1,200, Serotec, catalogue number STAR137F). The slides were rinsed three times in PBS and incubated with Alexa Fluor 594-conjugated species-specific secondary antibody for 1 h at room temperature (20–22 °C) (1/500, Invitrogen, catalogue number A11058) if needed. Slides were washed three times in...
PBS and stained with bis-benzimide/Hoechst (Invitrogen) to visualize nuclei and mounted with a 1:1 PBS-glycerol solution. Staining was visualized with a Zeiss Axiovert 200 microscope with an Axiocam MRM digital camera.

**Immunoblotting.** Protein was isolated from intestinal tissue segments of ~1 cm in 500 μl RIPA buffer with protease inhibitors with the Fastprep bead-beater system (MP Bio, BioSpec). Samples were subjected to four rounds of lysis at speed 6 for 20 s at 4 °C. Primary intestinal epithelial cells were lysed in Transwells with 50 μl RIPA buffer with protease inhibitors (Sigma). Total protein was quantified by a Pierce BCA Protein Assay Kit (Thermo Scientific). Supernatants from fecal samples and Transwells were taken as described above. Samples were run on SDS–polyacrylamide gel electrophoresis gels (AnykD or 7.5% Mini-Protean TGX gels, Bio-Rad) and transferred onto nitrocellulose membrane (Bio-Rad). Membranes were blocked with 3% milk in 0.1% Tween-20 Tris-buffered saline for 1 h at room temperature and probed with goat IgG anti-pIgR/secretory component (R&D, catalogue number AF2800) and rabbit IgG anti-Actin (Sigma, catalogue number 170-6515) before development with a SuperSignal West Dura chemiluminescent kit (Thermo Scientific). Immunoblots were quantified by ImageJ software. Whole-tissue pIgR/secretory component was normalized to actin, faecal samples were normalized by weight as described above, and apical supernatants were normalized by volume.

**Statistical analysis and experimental design.** No statistical methods were used to predetermine sample size. Statistical significance between two groups was determined by unpaired Student’s t-test if the data passed the D’Agostino–Pearson normality test or by Mann–Whitney U-test if the data did not pass the normality test. P value calculations were two-tailed. Comparison of more than two groups was performed with one-way ANOVA followed by Tukey’s multiple comparisons test, or two-way repeated measures ANOVA in Prism GraphPad software. Methods and P values are detailed in figure legends. Additional details of 16S rDNA analysis are included in the ‘16S rDNA Illumina sequencing and analysis’ section above. Letter n refers to the number of mice per group unless otherwise noted. All samples reflect unique biological replicates.

Inclusion of *in vitro* experiments was dependent upon expected performance of positive and negative controls. IgA ELISAs were performed blinded by a single investigator. Histological and immunofluorescence observations were performed blinded by two independent investigators. Samples were assessed in random order after being assigned numbers. Animals initially co-housed were randomly distributed to experimental groups, although no investigator blinding occurred during the execution of animal experiments.

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Extended Data Figure 1 | WT mice within two independent facilities exhibit binary faecal IgA levels, and the IgA-low phenotype is transferable between these facilities. a, Faecal IgA (normalized to faecal weight) from mice housed in either facility 1 (n = 28 IgA-high and n = 22 IgA-low mice) or facility 2 (n = 12 mice per group) was detected by anti-mouse IgA ELISA. b, c, WT IgA-high mice from one mouse facility were transplanted with homogenized faecal material from WT IgA-high or IgA-low mice from the other mouse facility, and faecal IgA was measured 14 days later by anti-mouse IgA ELISA. b, Facility 1 mice pre- (n = 18 mice) and post-faecal transplantation with facility 2 faecal samples (n = 8 post-IgA-high and n = 10 post-IgA-low mice). c, Facility 2 mice pre- (n = 10 mice) and post-faecal transplantation with facility 1 faecal samples (n = 4 post-IgA-high and n = 6 post-IgA-low mice). The dotted lines represent the limit of detection by ELISA. All values are mean ± s.e.m. One-way ANOVA: a, F = 44.59, P < 0.0001; b, F = 20.93, P < 0.0001; c, F = 12.92, P = 0.0004. Means with different footnote symbols are significantly different by Tukey’s multiple comparison test (P < 0.5).
Extended Data Figure 2 | IgA-high- and IgA-low-associated microbes can be stably passaged through \( pIgR^{-/-} \) recipients, and are vertically transmissible after recolonization. 

a, Schematic for repopulation of \( pIgR^{-/-} \) microbiota with WT IgA-high/IgA-low samples, followed by faecal transplantation (FT) of WT IgA-high/IgA-low samples to WT IgA-high mice. 

b, Faecal IgA on day 44 depicted in a. Mann–Whitney U-test: \( P = 0.0006, n = 8 \) mice per group. 

c, Experimental schematic of antibiotic treatment and transplant protocol for d and Fig. 1f, g. 

d, Faecal IgA of post-FT mice on day 30 pre-treated with metronidazole (Metro). One-way ANOVA: \( F = 6.525, P = 0.0012, n = 13 \) (pre-Metro), \( n = 15 \) (post-Metro), \( n = 8 \) (post-IgA-high FT), and \( n = 5 \) (post-IgA-low FT). All values are mean ± s.e.m. 

e, IgA-low mice converted to IgA-high from Fig. 1f were mated, and faecal IgA of their adult progeny was measured. One-way ANOVA: \( F = 18.29, P = 0.0002, n = 2 \) breeders, \( n = 10 \) progeny from four litters. Different footnote symbols indicate groups significantly different by Tukey’s multiple comparison test (\( P < 0.5 \)). 

Dotted lines: limit of detection.
Extended Data Figure 3 | DSS effects on pIgR−/− mice are dependent on IgA and not microbes. a, Faecal IgA levels were measured in WT mice from Fig. 2d, e after VNAM treatment and IgA-high/IgA-low faecal transplantation (FT), before the start of DSS treatment. Statistical analysis by Mann–Whitney U-test: $P = 0.0006$, $n = 7$ mice per group. b, Representative haematoxylin and eosin-stained histological sections of WT and pIgR−/− mice from Fig. 2d, e after 14 day VNAM treatment + IgA-high/IgA-low faecal transplantation. Representative of $n = 3$ (WT + IgA-high), $n = 6$ (WT + IgA-low), $n = 8$ (pIgR−/− + IgA-high), and $n = 10$ (pIgR−/− + IgA-low) mice. All values indicated as mean ± s.e.m. Means with different footnote symbols are significantly different by Tukey’s multiple comparison test ($P < 0.5$). Dotted lines, limit of detection.
Extended Data Figure 4 | Plasma cell numbers and pIgR expression are unchanged in the ileum and colon between IgA-high and IgA-low mice. a–d, Ileal and colonic sections from IgA-high and IgA-low mice were stained with anti-IgA (green) and bis-benzamide dye (blue); representative ×20 images are shown of n = 10 (a–c) or n = 9 mice (d). Scale bars, 100 μm. e, f, Quantification of ileal plasma cells per villus (e) and colonic plasma cells per ×20 field (area = 1.5 μm × 10^3 μm) (f) based on IgA staining. All values are mean ± s.e.m. Statistical analysis by Mann–Whitney U-test: e, P = 0.5191, n = 10 mice per group; f, P = 0.3117, n = 10 IgA-high and n = 9 IgA-low mice. g–j, Ileal and colonic sections from IgA-high and IgA-low mice were stained with anti-pIgR/secretory component (red) and bis-benzamide dye (blue); representative images are shown (n = 10 mice per group). Scale bars, 100 μm.
Extended Data Figure 5 | 16S rDNA sequencing identifies biomarkers for IgA-low and IgA-high samples. a, b, LEfSe analysis of 16S rDNA sequencing of IgA-low and IgA-high faecal samples from facilities 1 and 2 identified statistically significant bacterial taxa biomarkers for (a) IgA-low and (b) IgA-high samples. Biomarkers for facility 1 and biomarkers for facility 2 alone were identified by comparison of IgA-high and IgA-low samples within each facility. Biomarkers for facilities 1 and 2 were identified by comparison of all IgA-high and IgA-low samples from both facilities. No IgA-high biomarkers were identified when comparing all IgA-high and IgA-low samples from both facilities. Biomarkers for the indicated groups are plotted as taxonomic trees with GraPhlAn (http://huttenhower.sph.harvard.edu/graphlan); n = 13 (facility 1 IgA-high), n = 14 (facility 1 IgA-low), n = 73 (facility 2 IgA-high), and n = 68 (facility 2 IgA-low) samples. Statistical analysis is shown in Extended Data Table 1.
Extended Data Figure 6 | *Sutterella* is more abundant in IgA-low samples than IgA-high samples in both facilities. a–c, Relative abundance of sequences assigned by QIIME to the bacterial genus *Sutterella* from 16S rDNA analysis in (a) facility 1 and (b) facility 2. These results are summarized in c. One-way ANOVA: $F = 12.85, P < 0.0001$. $n = 13$ (facility 1 IgA-high), $n = 14$ (facility 1 IgA-low), $n = 73$ (facility 2 IgA-high), and $n = 68$ (facility 2 IgA-low) samples. Values in c are indicated as mean ± s.e.m. Means with different footnote symbols are significantly different by Tukey’s multiple comparison test ($P < 0.5$).
Extended Data Figure 7 | IgA-low cultured bacteria can degrade free secretory component in the absence of IgA, and secretory-component-degrading properties of these bacteria are active after freeze/thaw. Primary intestinal epithelial Transwell monolayers were pre-treated with 10 μM DAPT + 1 μg ml⁻¹ LPS on days 1 and 2 post-seeding to induce differentiation and plgR expression. Some wells were left untreated as negative controls. On day 3 post-seeding, either 3 μg of normal mouse dimeric IgA or media alone was added to the lower compartment of the Transwells. Different subsets of the DAPT + LPS-treated Transwells were also treated with one of the following conditions in the apical compartment: IgA-high/IgA-low bacterial cultures (pelleted bacterial or supernatant fraction), live or freeze/thawed IgA-high/IgA-low bacterial cultures (pelleted bacterial fraction). Apical Transwell supernatants were collected at 3 h and 6 h, and the amount of secretory component was measured by anti-secretory-component immunoblot. Representative anti-pIgR/secretory component and anti-actin immunoblots of intestinal epithelial monolayers at 6 h (one of three experiments). a, Secretory component degradation in the absence of IgA. b–d, Representative anti-secretory-component immunoblot and quantification of undegraded secretory component (denoted by the red brackets) at 3 h (c) and 6 h (d) over four independent experiments by ImageJ. e–g, Secretory component degradation by freeze/thawed bacterial cultures. Representative anti-secretory-component immunoblot and quantification of undegraded secretory component at 3 h (f) and 6 h (g) over five independent experiments by ImageJ. All values are mean ± s.e.m. One-way ANOVA: c, F = 1.834, P = 0.1831, n = 4 independent experiments with DAPT + LPS, no IgA repeated twice; d, F = 23.96, P = 0.0002, n = 4 independent experiments with DAPT + LPS, no IgA repeated twice; f, F = 7.444, P = 0.0045, n = 5 independent experiments with untreated and DAPT + LPS repeated three times; g, F = 31.53, P < 0.0001, n = 5 independent experiments with untreated and DAPT + LPS repeated three times. Means with different footnote symbols are significantly different by Tukey’s multiple comparison test (P < 0.5).
Extended Data Figure 8 | IgA-low cultured bacteria can degrade IgA.
Primary intestinal epithelial cell monolayers were pre-treated with 10 μM DAPT + 1 μg ml⁻¹ LPS on days 1 and 2 post-seeding to induce differentiation and pIgR expression. Some wells were left untreated as negative controls. On day 3 post-seeding, 3 μg of normal mouse IgA was added to the lower compartment of the Transwells. Different subsets of the DAPT + LPS-treated Transwells were also treated with combinations of the following in the apical compartment: live IgA-low bacterial cultures (either the pelleted bacterial or supernatant fraction), freeze/thawed IgA-low bacterial cultures, and a 1× protease inhibitor (PI) cocktail. Apical Transwell supernatants were collected at 3 h (a) and 6 h (b), and the amount of IgA was measured by anti-mouse IgA ELISA. The dotted lines represent the limit of detection by ELISA. All values are mean ± s.e.m. One-way ANOVA: a, F = 26.32, P < 0.0001, n = 8 (untreated), n = 8 (DAPT + LPS), n = 6 (IgA-low culture, pellet), n = 3 (IgA-low culture, supernatant), n = 4 (IgA-low culture, freeze/thawed), and n = 4 (IgA-low culture, + PI); b, F = 35.57, P < 0.0001, n = 8 (untreated), n = 8 (DAPT + LPS), n = 6 (IgA-low culture, pellet), n = 3 (IgA-low culture, sup), n = 3 (IgA-low culture, freeze/thawed), and n = 4 (IgA-low culture, + PI). Means with different footnote symbols are significantly different by Tukey’s multiple comparison test (P < 0.5); ND, not detected.
Extended Data Table 1 | 16S rDNA analysis of faecal samples from two facilities identified discriminant biomarkers between IgA-high and IgA-low samples

| Discriminant Biomarker | All Sample Comparison | Facility 1 Comparison | Facility 2 Comparison |
|------------------------|-----------------------|-----------------------|-----------------------|
|                        | LDA Effect Size (log 10) | P-value | IgA | LDA Effect Size (log 10) | P-value | IgA | LDA Effect Size (log 10) | P-value | IgA |
| Proteobacteria Betaproteobacteria | 3.37952 4.28E-17 | Low | 3.35270 0.004907 | Low | 3.43121 1.9E-15 | Low |
| Proteobacteria Betaproteobacteria Burkholderiales Alcaligenaceae Sutterella | 3.35757 4.28E-17 | Low | 3.35295 0.004907 | Low | 3.35954 1.9E-15 | Low |
| Bacteroidetes Bacteroidiales Bacteroidaceae | 3.81243 0.000368 | High | 3.46226 7.65E-05 | Low |
| Bacteroidetes Bacteroidiales Bacteroidiales Bacteroidaceae | 3.81243 0.000368 | High | 3.45797 7.65E-05 | Low |
| Bacteroidetes Bacteroidiales Prevotellaceae Prevotella | 3.46639 0.000856 | Low | 3.94789 6.31E-16 | Low |
| Bacteroidetes Bacteroidiales Rikenellaceae | 3.72308 0.015021 | High | 3.82644 1.5E-12 | Low |
| Bacteroidetes Bacteroidiales Enterobacteriales | 3.20358 0.000979 | Low | 3.13301 1.6E-12 | Low |
| Firmicutes Clostridia Clostridiales Christensenellaceae | 3.53769 0.015685 | Low | 3.33080 0.036150 | Low |
| Actinobacteria Coriobacteria Coriobacteriales Coriobacterium | 3.22514 0.004352 | Low |
| Bacteroidetes Bacteroidiales Paraprevotellaceae | 3.97592 9.65E-05 | Low |
| Bacteroidetes Bacteroidiales Paraprevotellaceae Prevotella | 3.97592 9.65E-05 | Low |
| Bacteroidetes Bacteroidiales Prevotellaceae | 3.82596 9.65E-05 | Low |
| Defibrinigens | 3.35837 0.000756 | Low |
| Deferribacteres Deferribacteres | 3.35874 0.000756 | Low |
| Deferribacteres Deferribacteres Delferribacterales | 3.36137 0.000756 | Low |
| Deferribacteres Deferribacteres Delferribacterales Deferribacterales | 3.35772 0.000756 | Low |
| Deferribacteres Deferribacteres Deferribacterales Deferribacterales Mucispirillum | 3.36082 0.000756 | Low |
| Proteobacteria Gammaproteobacteria | 3.30927 0.019810 | Low |
| Proteobacteria Gammaproteobacteria Enterobacteriales Enterobacteriales | 3.31707 0.019810 | Low |
| Firmicutes Clostridia Clostridiales | 4.57889 0.000680 | High |
| Firmicutes Clostridia Clostridiales Lachnospiraceae | 3.82545 0.017366 | High |
| Firmicutes Clostridia Clostridiales Lachnospiraceae Dorea | 3.13299 0.026976 | High |
| Firmicutes Clostridia Clostridiales Ruminococcaceae Oscillospira | 3.84527 0.000447 | High |
| Firmicutes Clostridia Clostridiales Ruminococcaceae Ruminococcus | 3.77812 0.000499 | High |
| Proteobacteria Alphaproteobacteria RF32 | 3.27951 0.000611 | High |
| Proteobacteria Epsilonproteobacteria Campylobacteraceae Helicobacteraceae | 3.65250 0.000393 | High |
| Tenericutes | 3.24743 0.000106 | High |
| Tenericutes Mollicutes | 3.25353 0.000120 | High |
| Tenericutes Mollicutes RF32 | 3.25469 0.000120 | High |
| Bacteroidetes | 4.70727 0.000397 | Low |
| Bacteroidetes Bacteroides | 4.70727 0.000397 | Low |
| Bacteroidetes Bacteroides Bacteroidiales Odoribacteraceae | 3.41723 1.22E-18 | Low |
| Bacteroidetes Bacteroides Bacteroidiales Odoribacteraceae Odoribacter | 3.41723 1.22E-18 | Low |
| Bacteroidetes Bacteroidiales Porphyromonadaceae | 3.11464 0.000676 | Low |
| Bacteroidetes Bacteroidiales Porphyromonadaceae Parabacteroides | 3.06586 0.000676 | Low |
| Bacteroidetes Bacteroidiales S24_7 | 4.49336 0.034288 | High |
| Firmicutes Clostridia Clostridiales Dehalobacteriaceae | 3.44482 0.010761 | Low |
| Firmicutes Clostridia Clostridiales Dehalobacteriaceae Dehalobacterium | 3.45906 0.010761 | Low |
| Proteobacteria | 3.38346 8.96E-13 | Low |
| TM7 | 3.94036 0.013973 | Low |
| TM7 TM7 3 | 3.79262 0.013973 | Low |
| TM7 TM7 3 CWGD0 | 3.96516 0.013973 | Low |
| TM7 TM7 3 CWGD0 F16 | 3.84749 0.013973 | Low |
| Cyanobacteria | 3.19671 0.000962 | High |
| Cyanobacteria 4C0d_2 | 3.38513 0.001431 | High |
| Cyanobacteria 4C0d_2 Y2S | 3.31490 0.001431 | High |
| Firmicutes | 4.64404 0.003323 | High |
| Firmicutes Clostridia Clostridiales Clostridiales | 3.93227 2.01E-05 | High |
| Firmicutes Clostridia Clostridiales Clostridiales 02d06 | 2.56784 0.024838 | High |
| Firmicutes Clostridia Clostridiales Clostridiales Clostridium | 2.54472 0.019153 | High |
| Firmicutes Clostridia Clostridiales Clostridiales Sarcina | 2.56391 0.021179 | High |
| Firmicutes Clostridia Clostridiales Peptococcaceae ro4_4 | 3.26329 0.000457 | High |
| Firmicutes Clostridia Clostridiales Peptostreptococcaceae | 2.74357 0.034900 | High |
| Firmicutes Clostridia Clostridiales Ruminococcaceae | 3.68674 0.000262 | High |
| Firmicutes Erysipelotrichi Erysipelotrichales Erysipelotrichaceae | 2.56710 0.014173 | High |
| Tenericutes Mollicutes Anaeroplasmatales | 3.43058 0.012537 | High |
| Tenericutes Mollicutes Anaeroplasmatales Anaeroplasmatales | 3.43172 0.012537 | High |
| Tenericutes Mollicutes Anaeroplasmatales Anaeroplasmatales Anaeroplasmatales | 3.43713 0.012537 | High |

The relative abundance of operational taxonomic units from 16S rDNA amplification of faecal sample DNA was determined with QIIME, and these data were input into LEfSe to identify discriminant biomarkers. After Kruskal–Wallis analysis (with an α value of 0.05) of all features, a linear discriminant analysis model was used to rank discriminant features by the effect size with which they differentiated classes, in this case IgA-high versus IgA-low samples. We performed analysis to discriminate between IgA-high and IgA-low samples from both facilities ("All Sample Comparison"), from facility 1 only ("Facility 1 Comparison") or facility 2 only ("Facility 2 Comparison"). Biomarkers are depicted as phylum.class.order.family.genus, to the level to which the taxonomic units were assignable by QIIME. "IgA" indicates whether the biomarker was enriched in IgA-high samples ("High") or IgA-low samples ("Low").
Extended Data Table 2 | IgA-low sample cultures enrich for unique bacterial taxa compared with cultures from IgA-high samples

| Bacterial Taxa                        | Mean and Standard Deviation | One-way analysis of variance | Tukey’s multiple comparisons test |
|--------------------------------------|-----------------------------|-----------------------------|----------------------------------|
|                                      | Pre IgA-high                | Post IgA-high               | Pre IgA-high vs. Post IgA-low | Pre IgA-high vs. Pre IgA-low | Pre IgA-high vs. Post IgA-low | Pre IgA-low vs. Post IgA-low |                                      |
| Bacteroidales, Bacteroidaceae.       |                            |                             |                                 |                               |                             |                                 |                                      |
| Bacteroides                          | 0.0007 ± 0.0005            | 0.0001 ± 0.0001             | 0.0535 ± 0.0122                 | 0.1791 ± 0.0127               | F = 367.9                  | P < 0.0001                     | NS                                 |
|                                      |                            |                             |                                 |                               |                             |                                 |                                      |
| Bacteroidales, Porphyromonadaceae.   | 0.0002 ± 0.0003            | 0.0001 ± 0.0001             | 0.0015 ± 0.0001                 | 0.0173 ± 0.0034               | F = 80.90                  | P < 0.0001                     | NS                                 |
| Parabacteroides                      |                            |                             |                                 |                               |                             |                                 |                                      |
| Bacteroidales, Prevotellaceae.       | 0 ± 0                      | 0 ± 0                       | 0.0657 ± 0.0343                 | 0.0002 ± 0.0003               | F = 14.66                  | P = 0.0003                     | NS                                 |
| Prevotella                           |                            |                             |                                 |                               |                             |                                 |                                      |
| Bacteroidales, S24-7. Unclassified. | 0.8166 ± 0.0809            | 0.1087 ± 0.0164             | 0.7920 ± 0.0088                 | 0.0905 ± 0.0377               | F = 319.0                  | P < 0.0001                     | NS                                 |
| Sutterella                           |                            |                             |                                 |                               |                             |                                 |                                      |
| Burkholderiales, Alcaligenaceae.     | 0.0006 ± 0.0003            | 0.0004 ± 0.0002             | 0.0241 ± 0.0067                 | 0.4037 ± 0.0303               | F = 660.5                  | P < 0.0001                     | NS                                 |
| Sutterella                           |                            |                             |                                 |                               |                             |                                 |                                      |
| Clostridiales, Unclassified.         | 0.1665 ± 0.0850            | 0.0019 ± 0.0014             | 0.0264 ± 0.0007                 | 0.0002 ± 0.0002               | F = 13.75                  | P = 0.0003                     | ***                                |
| Unclassified                         |                            |                             |                                 |                               |                             |                                 |                                      |
| Clostridiales, Lachnospiraceae.      | 0.0007 ± 0.0004            | 0.0012 ± 0.0001             | 0.0001 ± 0.0007                 | 0.0014 ± 0.0001               | F = 3.717                  | P = 0.0423                     | NS                                 |
| Unclassified                         |                            |                             |                                 |                               |                             |                                 |                                      |
| Clostridiales, Mogibacteraeae.       | 0.0004 ± 0.0851            | 0.0753 ± 0.0007             | 0.0007 ± 0.007                  | 0.0674 ± 0.0047               | F = 3.021                  | P = 0.0716                     | NS                                 |
| Unclassified                         |                            |                             |                                 |                               |                             |                                 |                                      |
| Enterobacteriales, Enterobacteriaceae.| 0.0003 ± 0.0004            | 0.0004 ± 0.0004             | 0.0004 ± 0.0004                 | 0.1760 ± 0.0152               | F = 531.4                  | P < 0.0001                     | NS                                 |
| Unclassified                         |                            |                             |                                 |                               |                             |                                 |                                      |
| Enterobacteriales, Proteus           | 0.014 ± 0.0793             | 0.0015 ± 0.0006             | 0.0322 ± 0.0019                 | NS                             | F = 360.4                  | P < 0.0001                     | NS                                 |
| Lactobacillaceae, Lactobacillus.     | 0.0127 ± 0.0176            | 0.0249 ± 0.0245             | 0.0339 ± 0.0245                 | 0.0319 ± 0.0048               | F = 1.510                  | P = 0.2622                     | NS                                 |

Means and standard deviations of assigned order, family, genus operational taxonomic units are depicted. Each taxon was compared between groups by one-way ANOVA followed by Tukey’s multiple comparisons test (P < 0.5).