Enrichment of intestinal *Lactobacillus* by enhanced secretory IgA coating alters glucose homeostasis in *P2rx7−/−* mice

Lisa Perruzza¹, Francesco Strati¹, Giorgio Gargari², Anna Maria D’Erchia², Bruno Fosso³, Graziano Pesole¹,³,⁴, Simone Guglielmetti² & Fabio Grassi¹,⁵,⁶

The secretory immunoglobulin A (SIgA) in mammalian gut protects the organism from infections and contributes to host physiology by shaping microbiota composition. The mechanisms regulating the adaptive SIgA response towards gut microbes are poorly defined. Deletion of *P2rx7*, encoding for the ATP-gated ionotropic P2X7 receptor, leads to T follicular helper (Tfh) cells expansion in the Peyer’s patches (PPs) of the small intestine, enhanced germinal centre (GC) reaction and IgA secretion; the resulting alterations of the gut microbiota in turn affects host metabolism. Here, we define gut microbiota modifications that correlate with deregulated SIgA secretion and metabolic alterations in *P2rx7−/−* mice. In particular, *Lactobacillus* shows enhanced SIgA coating in *P2rx7−/−* with respect to wild-type (WT) mice. The abundance of SIgA-coated lactobacilli positively correlates with Tfh cells number and body weight, suggesting *Lactobacillus*-specific SIgA response conditions host metabolism. Accordingly, oral administration of intestinal *Lactobacillus* isolates from *P2rx7−/−* mice to WT animals results in altered glucose homeostasis and fat deposition. Thus, enhanced SIgA production by P2X7 insufficiency promotes *Lactobacillus* colonization that interferes with systemic metabolic homeostasis. These data indicate that P2X7 receptor-mediated regulation of commensals coating by SIgA is important in tuning the selection of bacterial taxa, which condition host metabolism.

The intestinal microbiota influences host physiology, metabolism and immune system homeostasis¹. After birth, microbial colonization stimulates the development of host's gut-associated lymphoid tissues (GALT), including cryptopatches, Peyer patches (PPs), and isolated lymphoid follicles². The ensuing interaction between microbes and immune system results in the relative immune tolerance of the commensal microbial community and selection of beneficial taxa³. The secretory immunoglobulin A (SIgA) contributes to the establishment of the intestinal barrier by controlling binding of bacteria to the epithelium and the possible translocation of pathobionts into the lamina propria⁴. However, it is not yet completely understood which members of the gut microbiota are actually targeted by SIgA and which contribution IgA-coated bacteria might provide to host physiology. Although SIgA-coated bacteria have been described to be enriched in taxa with potential pro-inflammatory properties conferring susceptibility to colitis⁵ and weight loss⁶, IgA-coated bacteria from healthy humans protect mice from disease⁷ and are important for the preservation of commensal diversity and community networks in the human gut⁸. Recently, a regulatory mechanism whereby SIgA would foster mucosal colonization of the human gut commensal *Bacteroides fragilis* has been described⁹. Furthermore, SIgA-coated *Bacteroides thetaiotaomicron* induced the expression of Mucus-Associated Functional Factors (MAFFs) that regulated the composition and metabolic function of the whole gut microbiota by promoting symbiosis with members of the phylum *Firmicutes* and

¹Institute for Research in Biomedicine, Faculty of Biomedical Sciences, Università della Svizzera Italiana (USI), 6500, Bellinzona, Switzerland. ²Department of Food, Environmental, and Nutritional Sciences (DeFENS), Università degli Studi di Milano, 20133, Milan, Italy. ³Institute of Biomembranes and Bioenergetics, National Research Council, 70126, Bari, Italy. ⁴Department of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari, 70126, Bari, Italy. ⁵Department of Medical Biotechnology and Translational Medicine, Università degli Studi di Milano, 20129, Milan, Italy. ⁶Istituto Nazionale Genetica Molecolare "Romeo ed Enrica Invernizzi", 20122, Milan, Italy. Lisa Perruzza and Francesco Strati contributed equally. Correspondence and requests for materials should be addressed to F.G. (email: fabio.grassi@irb.usi.ch)

Received: 1 February 2019
Accepted: 11 June 2019
Published online: 27 June 2019
The vast majority of SIgA-coated bacteria resides in the small intestine and is targeted by T cell-independent antibodies; only a minority of commensals would be responsible for eliciting T cell-dependent SIgA responses. T follicular helper (Tfh) cells in PPs are essential for SIgA affinity maturation that in turn modulates the structure and function of the intestinal microbiota. Adenosine triphosphate (ATP) is a ubiquitous extracellular messenger, which activates purinergic receptors in the plasma membrane of eukaryotic cells termed P2X and P2Y receptors. The ATP-gated ionotropic receptor P2X7 is a signature gene of effector T cell subsets and is selectively upregulated in Tfh cells of PPs. In mice with deletion of the P2rx7 gene, Tfh cells are expanded in PPs because of resistance to cell death induced by extracellular ATP (eATP). The altered control of Tfh cells by defective sensing of microbiota derived ATP leads to enhanced secretion of T cell dependent IgA and increased frequency of replacement mutations in the IgVH1 family's complementarity determining region (CDR) suggesting enhanced affinity maturation of IgA responses. Therefore, eATP modulates adaptive IgA responses to ensure physiological mucosal colonization. Furthermore, the alteration of the gut microbiota due to the lack of P2X7 mediated control of Tfh cells results in dysregulated metabolic homeostasis, consistent with the central role of SIgA in regulating host-microbiota interactions and host physiology. A number of studies in mice and humans have demonstrated that obesity is associated with alterations of the gut microbiota. Intestinal dysbiosis has been suggested to play a causal role in the development of insulin resistance as well as inflammation and macrophage accumulation in adipose tissue. Recently, a genome-wide association study has shown the association of hypo-functioning P2X7 variants with impaired glucose homeostasis and obesity in humans. Here, the characterization of the faecal microbiota targeted by SIgA in P2rx7−/− mice allowed us to identify the enhanced SIgA coating of Lactobacillus as a possible mechanism contributing to the observed metabolic disturbance.

**Results**

**P2rx7−/− mice show altered metabolic parameters and enhanced Tfh cells activity.** P2rx7−/− mice are characterized by altered fat distribution. In these mice, dysregulated Tfh cells activity with consequent enhanced GC reactions and secretion of high affinity IgA affects microbiota composition resulting in altered glucose homeostasis and fat deposition. White adipose tissue (WAT), body weight and blood glucose were increased in P2rx7−/− mice with respect to WT littermates (p < 0.01, Wilcoxon rank-sum test; Fig. 1a–c). Food...
consumption and energy harvesting were not different between the two strains of mice. However, the daily profile of fuel metabolism, as measured by the respiratory exchange ratio (RER), the ratio of consumed oxygen to produced carbon dioxide, showed differences between the two groups of mice. P2rx7−/− mice showed lower RER values during the inactive (light) phase with respect to WT littermates, suggesting lower energy expenditure might at least partially contribute to the body weight increase in these mice (Fig. 1g). As expected, the proportions of Th and GC B cells were significantly higher in P2rx7−/− mice than in WT littermates (p < 0.01, Wilcoxon rank-sum test; Fig. 1d,e) as well as the percentage of IgA-coated bacteria (p < 0.0001, Wilcoxon rank-sum test; Fig. 1f).

**P2rx7−/− mice harbour an altered gut microbiota and enhanced IgA response towards bacteria residing in the small intestine.** In order to define possible differences in bacterial IgA coating between P2rx7−/− mice and WT littermates, we characterized the IgA+ and IgA− fractions of the faecal microbiota through high-throughput sequencing of the V5–V6 region of the 16S rRNA gene (IgA-SEQ) (Fig. S1). The analysis of alpha-diversity (i.e. the within samples diversity) revealed no significant differences between the IgA+ or IgA− fractions of P2rx7−/− and WT mice. Nevertheless, we observed a reduction close to statistical significance, of alpha-diversity in the presorted samples from P2rx7−/− mice compared to WT controls (p = 0.055 on the Inverse Simpson index; Wilcoxon rank-sum test), in agreement with previous observations on the gut microbiota of obese mice and humans.

We then characterized the microbial community structure of the IgA+, IgA− and presorted faecal microbiota through beta-diversity analysis on the unweighted UniFrac distance and Bray-Curtis dissimilarity. The faecal microbiota of P2rx7−/− mice clustered apart from that of WT littermates as well as the IgA− fraction (p < 0.05, PERMANOVA on the Bray-Curtis dissimilarity, Table 1 and Fig. 2a), suggesting that enhanced IgA secretion due to lack of Th cells control via P2X7 has a significant effect on the composition of the gut microbiota (Figs 2b and S2a). Phylum level analysis showed a significant increase in the Firmicutes/Bacteroidetes ratio in the P2rx7−/− IgA+ microbiota (p = 0.03, Wilcoxon rank-sum test, Fig. 2c) due to the significantly higher relative abundance of Firmicutes (mean relative abundance, 65.6% in P2rx7−/−, 27.8% in WT), as confirmed by LEfSe analysis (Fig. 3a). On the contrary, we did not detect significant differences in the Firmicutes/Bacteroidetes ratio of the IgA− microbiota (Fig. 2d) and presorted faecal microbiota of P2rx7−/− and WT mice (Fig. S2b). The Firmicutes/Bacteroidetes ratio is a rough estimator of intestinal dysbiosis and its increase has been associated to obesity and metabolic abnormalities in humans and mice.

The enhanced SlgA response due to lack of P2X7 receptor resulted in the enhanced SlgA coating and enrichment of bacterial taxa that usually inhabit the small intestine i.e. Lactobacillus, Enterococcus and Enterobacteriaceae; however, anti-inflammatory and anti-obesogenic taxa such as Akkermansia and Prevotella24,25 were depleted from the SlgA+ microbiota (LEfSe, p < 0.05, Wilcoxon rank-sum test, LDA > 2.0; Fig. 3a). Lactobacillus was the most abundant genus within the P2rx7−/− IgA+ microbiota (mean relative abundance, 44.1% in P2rx7−/−, 14.1% in WT, Fig. 2b) and was highly enriched in this fraction (Fig. 3c). In the IgA− microbiota of P2rx7−/− mice, we observed a significant increase in the relative abundance of Lachnospiraceae, Bifidobacteria and Ruminococcaceae (LEfSe, p < 0.05, Wilcoxon rank-sum test, LDA > 2.0; Fig. 3b), whereas the presorted faecal microbiota of P2rx7−/− mice was depleted of bacterial taxa important for intestinal homeostasis, e.g. Barnesiella, Ruminococcaceae, Clostridium cluster IV26,27 (LEfSe, p < 0.05, Wilcoxon rank-sum test, LDA > 2.0; Fig. S2c). Notably, the genus Turicibacter, enriched in the IgA− fraction of P2rx7−/− mice (Fig. 3c), was exclusively present in these mice (Figs 2b and S2c). Altogether, these data suggest SlgA response in P2rx7−/− mice conditions intestinal microbial ecology beyond bacterial taxa that are selectively targeted by SlgA.

**Correlation of gut microbes conditioned by enhanced SlgA response with metabolic and immunological parameters in P2rx7−/− mice.** The relationship between metabolic disorders and gut microbiota has been widely established, as well as the role of the immune system and Th cells activity in selecting a beneficial microbiota for host metabolism. The enhanced Th cells activity in P2rx7−/− mice was accountable for gut microbiota alterations in both IgA+ and IgA− fractions. To evaluate which bacterial taxa might be important for energy metabolism and regulation of mucosal immunity via P2X7, we correlated metabolic and immunologic parameters with the most abundant bacterial genera retrieved by IgA-SEQ. The genus Lactobacillus, within the IgA+ microbiota of P2rx7−/− and WT mice, positively correlated with body weight and abundance of Tlh cells in PPs (Fig. 4a,b). On the other hand, we observed negative correlations of Tlh cells, GC B cells, blood glucose, body and WAT weight with the genera Prevotella, Bacteroides and Barnesiella (Fig. 4a,b). Thus, modified SlgA targeting of these genera by deregulated T follicular help in P2rx7−/− mice could contribute to host metabolic alterations. Within the IgA− microbiota of P2rx7−/− and WT animals, the relative abundance of unclassified Lachnospiraceae

| Metric | Unweighted UniFrac | Bray-Curtis | Unweighted UniFrac | Bray-Curtis | Unweighted UniFrac | Bray-Curtis | p-value |
|--------|--------------------|-------------|--------------------|-------------|--------------------|-------------|---------|
| IgA+   | 1.517              | 0.159       | 0.175              |             | 2.611              | 0.246       | 0.046   |
| WT vs P2rx7−/− | Bray-Curtis | 1.259 | 0.135 | 0.197 | 2.220 | 0.217 | 0.054 |
| Pre-sorted | Unweighted UniFrac | 1.285 | 0.138 | 0.005 | 1.765 | 0.180 | 0.043 |

Table 1. Permutational multivariate analysis of variance (PERMANOVA) of the IgA+, IgA− and pre-sorted faecal microbiota in WT vs P2rx7−/− mice according to the unweighted UniFrac distance and Bray-Curtis dissimilarity.
positively correlated with body weight, blood glucose and Tfh cells while 
*Prevotella* and *Bacteroides* negatively correlated with body weight and % of GC B cells (Fig. 4c,d), consistent with previous observations on the high 
relative abundance of *Lachnospiraceae*, and low abundance of *Bacteroides* and *Prevotella* in obese individuals and 
mice25,28,29. Moreover, significantly increased *Lachnospiraceae* were found in the caecal microbiota of 
P2rx7−/− mice16. Finally, different taxa (*i.e.*, *Alistipes*, *Oscillibacter*, *Mucispirillum*, *Clostridium XIVb*, unclassified genera of 
*Clostridiales* and *Ruminococcaceae*) in the WT and 
P2rx7−/− faecal microbiota negatively correlated with body 
and WAT weight, blood glucose and GC B cells (Fig. S2d,e). Altogether, these data suggest that P2X7 activity in 
Tfh cells conditions microbiota composition and host metabolism via regulated SIgA targeting of selected bacte-
rial genera that in turn might affect metabolically relevant taxa independently of SIgA targeting.

*Intestinal Lactobacillus isolates from P2rx7−/− mice alter glucose metabolism in wild-type animals.* The genus *Lactobacillus* was significantly enriched in the IgA+ fraction of the P2rx7−/− microbiota (Fig. 3a,c) and correlated with the metabolic and immune phenotype of P2rx7−/− mice (Fig. 4a,b). Different spe-
cies of lactobacilli have been associated with body weight gain30 and juvenile growth rate31 through the increase of 
dietary protein digestion and amino acid intake by the host32. Quantification of the genus *Lactobacillus* by qPCR 
in samples from small intestine, caecum and faeces confirmed the significant enrichment of lactobacilli in the gut 
of P2rx7−/− mice (Fig. 5a). To investigate whether lactobacilli may contribute to the metabolic alterations induced 
by non-functional P2X7 receptor16, we recovered from the gastrointestinal tract of P2rx7−/− mice different iso-
lates that were all belonging to the species *L*. *murinus* and *L*. *reuteri*. Accordingly, we detected increased titres of 
faecal IgA specific for these *Lactobacillus* species in P2rx7−/− as compared to WT mice (Fig. S3c). Conversely, IgA 
coating of small intestine microbes by faecal IgA derived from either WT or P2rx7−/− mice was undistinguishable 
(Fig. S3a,b), suggesting SIgA response in the small intestine of P2rx7−/− mice is skewed toward lactobacilli.

We administered by oral gavage for three weeks the isolates *L*. *murinus* SI1/6 and *L*. *reuteri* SI1/3 from P2rx7−/− 
mice to specific pathogen-free (SPF) mice that were depleted of endogenous microbiota by antibiotics (SPF-Abx).
Treatment of SPF-Abx mice with both *Lactobacillus* isolates induced a significant increase of glycaemia compared to non-treated mice or animals gavaged with *E. coli* (Fig. 5b), altered glucose homeostasis with reduced glucose clearance in the glucose tolerance test (GTT) (Fig. 5d,e) as well as increased perigonadal fat deposition (Fig. 5c). The altered metabolic homeostasis observed in *Lactobacillus*-treated mice was unrelated to Tfh (% Tfh cells: CTRL, 7.77 ± 2.4; *E. coli* DH10B, 11.2 ± 4.2; *L. murinus*, 9.56 ± 2.1; *L. reuteri*, 9.43 ± 3.3) or GC B (% GC B cells: CTRL, 9.91 ± 2.4; *E. coli* DH10B, 9.82 ± 2.4; *L. murinus*, 11.8 ± 2.1; *L. reuteri*, 11.1 ± 1.9) cells abundance in PPs. Furthermore, oral gavage of *Lactobacillus* into *Igh-J*−/− mice, which carry a deletion in the J region of the Ig heavy chain locus and lack SIgA, showed similar alterations in glucose metabolism to *Lactobacillus*-treated WT animals (Fig. S4) suggesting that SIgA were important in enriching *Lactobacillus* in the intestine of *P2rx7*−/− mice but not necessarily required for inducing the observed metabolic alterations.

**Discussion**

Intestinal homeostasis requires a balanced microbiota, which is also shaped in structure and functions by secreted IgA. IgA coating identifies bacterial taxa with the potential ability to interact with the host and colonize the intestinal mucosa; in addition, it can influence bacterial gene expression, metabolism and ability to colonize different intestinal ecological niches. Since P2X7 deficiency leads to enhanced secretion of intestinal IgA and alterations of both gut microbiota and host metabolism, the *P2rx7*−/− mouse represents a unique model for the study of the role of SIgA in the remodelling of gut microbiota and metabolic homeostasis. In fact, the enhanced production of SIgA resulted in increased SIgA coating of bacteria typically residing in the small intestine, especially *Lactobacillus*, *Enterococcus* and *Enterobacteriaceae*. The *P2rx7*−/− SIgA+ microbiota was characterized by a significant increase of the *Firmicutes/Bacteroidetes* ratio, a common feature of obese mice and humans, suggesting that enhanced SIgA-coating could enrich bacterial taxa contributing to metabolic alterations. The genus *Lactobacillus*, belonging to the phylum *Firmicutes*, has been associated with body weight gain, obesity and modulation of SIgA production, although a consensus regarding its role in health and disease has not been fully achieved. In *P2rx7*−/− mice, we observed a

**Figure 3.** The lack of P2X7 receptor alters the gut microbiota at different taxonomic levels. (a,b) Log<sub>10</sub> of LDA scores for the most discriminant bacterial taxa identified by LEfSe in the IgA+ (a) and IgA− (b) fractions of faecal microbiota from *P2rx7*−/− and WT mice. Only taxa having a p < 0.05 (Wilcoxon rank-sum test) and LDA > 2[0] are shown. (c) Enrichment of indicated taxa (with relative abundance >0.1%) in the IgA+ and IgA− fractions of *P2rx7*−/− mice and WT littermate controls. Error bars indicate the standard error.
positive correlation between the relative abundance of IgA⁺ *Lactobacillus* with body weight as well as with the abundance of Tfh cells in PPs. Consistent with a direct causal role of enriched lactobacilli in contributing to the metabolic phenotype of *P2rx7⁻/⁻* mice, the administration of two *Lactobacillus* isolates from *P2rx7⁻/⁻* to WT mice.
or Igh-J−/− mice reproduced the impaired glucose metabolism observed in P2rx7−/− mice. These experiments suggest that purinergic regulation of adaptive SIgA response in GALT can modulate intestinal colonization by commensals, which affect host physiology.

A physiological bacterial IgA coating regulated by T follicular regulatory (Tfr) cells and P2X7 proficient Tfh cells, contributes to the maintenance of a well-balanced intestinal microbial community within different ecological niches. Specific changes in the IgA+ and IgA− microbiota of P2rx7−/− mice correlate with dysmetabolic features of these animals. How SlgA controls the diversification and balance of the gut microbiota is not yet clearly understood; our work sheds light on the importance of the regulation of T cell dependent SIgA via the eATP/P2X7 axis in controlling the abundance of bacterial taxa, such as Lactobacillus, that can affect host metabolic homeostasis.

In conclusion, by analysing mice deficient in the ATP-gated ionotropic P2X7 receptor, which limits Tfh cells in the PPs and adaptive SIgA production, we positively correlated Tfh cells number and body weight with increased SIgA coating and enrichment of lactobacilli. We hypothesize the eATP/P2X7 axis constitutes a crucial

Figure 5. Altered glucose homeostasis and fat deposition in Lactobacillus treated WT animals. (a) Absolute quantification of the genus Lactobacillus by qPCR in the small intestine (SI), caecum and faeces of P2rx7−/− (yellow) and WT (blue) mice. (b) Glycaemia after 21 days of treatment. (c) Fat deposition as measured by perigonadal white adipose tissue weight. (d) Glucose tolerance test (GTT). (e) Areas under the curve (AUC) of GTT. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, Wilcoxon rank-sum test; n = 10 per group; n = 7 for GTT of L. murinus group.
regulatory pathway in Tfh cells to ensure controlled S IgA coating and abundance of commensals which affect host metabolism.

Materials and Methods

Mice and in vivo experiments. C57BL/6J, P2rx7+/− (B6.129P2-P2rx7tm1Gab/J) and Igh-J−/− (B6.129P2-Igh-Jtm1Cgn/J) mice from Jackson Lab were bred in the specific pathogen-free (SPF) facility at the Institute for Research in Biomedicine, Bellinzona, Switzerland. The colonies of C57BL/6J, P2rx7+/− and Igh-J−/− were maintained onsite with heterozygous breeders and littermates kept in the same cages until weaning at 4 week of age. Animals were housed in ventilated cages in a 12 h light/dark cycle, with free access to water and standard autoclaved chow. Food intake was measured using metabolic cages. For the in vivo Lactobacillus administration experiments, 4 weeks old C57BL/6J and Igh-J−/− animals were treated with an antibiotic mixture containing Vancomycin (1.25 mg), Ampicillin (2.5 mg) and Metronidazole (1.25 mg) (VAM) in 200 μl water per mouse by oral gavage for 7 days to promote a more efficient bacterial colonization. Later, these animals were given 5 × 10^9 CFU of Lactobacillus reuteri, Lactobacillus murinus or E. coli DH10B by oral gavage in 200 μl PBS for 21 days. Glucose tolerance test was performed as follow: animals were fasted for 12 h and then received an intraperitoneal injection of glucose (2 g/kg of body weight). Blood glucose was measured using a glucometer (Healthpro-X1, Axapharm) on samples collected from tail vein. For RER measurement, mice were transferred to single housing in Phenomaster System (TSE Systems Gmbh, Bad Homburg, Germany) one day before the study start for acclimatization, followed by two days of continued measurements. During the study period, air flow, temperature, oxygen and carbon dioxide content, oxygen uptake (VO₂), carbon dioxide production (VCO₂) were measured simultaneously using standard indirect calorimetry analysis. Respiratory exchange ratio was calculated automatically from VO₂ and VCO₂. Data were collected in TSE Phenomaster software and exported to excel. For ex vivo experiments, mice were euthanized by CO₂ inhalation and Peyer’s patches, white perigonadal adipose tissue and faeces, small intestine of C57BL/6 and C57BL/6J, P2rx7+/− mice. Tfh and GC B cells were stained with labelled antibodies diluted in PBS with 2% heat-inactivated foetal bovine serum (FBS) for 20 min on ice. The following mouse antibodies (mAbs) were purchased from BD Biosciences (BD Biosciences, Franklin Lakes NJ, USA): biotin conjugated anti-CXCR5 (clone: 2G8, Cat. #: 551960), PE conjugated anti-Fas (clone: Jo2 Cat. #: 554258), PE conjugated anti-ICOS (clone: 7E.17G9, Cat. #: 552146). The following mAbs were purchased from Biolegend (Biolegend, San Diego, CA, USA): APC conjugated anti-PD-1 (Clone: RMPI-30, Cat. #: 109111), APC conjugated anti-B220 (clone: RA3-6B2, Cat. #: 103212), PE-Cy7 conjugated anti-CD4 (Clone: GK1.5, Cat. #: 100422), APC-Cy7 conjugated anti-CD19 (clone: 6D5, Cat. #: 115530), APC conjugated streptavidin (Cat. #: 405207). The following mAbs were purchased from eBioscience (eBioscience, Santa Clara, CA, USA): Percp-eFluor710 conjugated anti-CD3 (Clone: 17A2, Cat. #: 46-0032-80) and efluor405 conjugated streptavidin (Cat. #: 48-4317-82). Fluorescein labelled Peanut Agglutinin (PNA) (Cat.#: FL-10-71) was purchased from Vectorlabs (Vector Laboratories, Burlingame, CA, USA). Fluorescein Isothiocyanate (FITC) conjugated anti-IgA (Cat. #: 1040-02) and biotinylated anti-mouse IgA (Cat. #: 1040-08) were obtained from Southern Biotech. SYTO BC Green Fluorescent Nucleic Acid Stain (Cat. #: S34855) was purchased from Thermo Fisher Scientific. Samples were acquired on an LSFRFortessa (BD Biosciences, Franklin Lakes NJ, USA) flow cytometer. Data were analysed using the FlowJo software (TreeStar, Ashland, OR, USA) or FACS Diva software (BD Biosciences, Franklin Lakes NJ, USA).

Faecal IgA flow cytometry and sorting of IgA+ and IgA− bacteria. For analysis of IgA coated bacteria in flow cytometry, fresh faecal pellets were collected into sterile 2 mL Eppendorf tubes and homogenized in PBS (0.1 g/ml). The homogenized samples were centrifuged at 400 × g for 5 min to remove larger particles from bacteria. Supernatants were centrifuged at 8,000 × g for 10 min to remove unbound IgAs. Pellet bacteria were resuspended in PBS 5% goat serum (Jackson ImmunoResearch, West Grove, PA, USA), incubated 15 min on ice, centrifuged and resuspended in PBS 1% BSA for staining with APC conjugated rabbit anti-mouse IgA antibodies (Cat. #: SAB1186; Brookwood Biomedical, Birmingham, AL, USA). After 30 min incubation, bacteria were washed twice and resuspended in 2% paraformaldehyde in PBS for acquisition at LSFRFortessa. For both analysis and sorting of the IgA+ and IgA− fractions at FACSAria, forward and side scatter parameters were used in logarithmic mode. SYTO BC was added to identify bacteria-sized particles containing nucleic acids. Rag1−/− mice were used as control for absence of Igs-coated bacteria.

Determination of binding of faecal IgA to small intestine microbiota and titers of faecal IgA specific for lactobacilli. Binding of faecal IgA to small intestine microbiota and titers of lactobacilli specific IgA in faecal samples were measured by flow cytometry. To detect the binding of faecal IgA to small intestine microbiota, the intestinal content of C57BL/6 and P2rx7+/− mice was collected and homogenized in PBS (0.1 g/ml). The homogenized samples were centrifuged at 400 × g for 5 min to remove larger particles from bacteria. Supernatants were then centrifuged at 20,000 × g for 10 min to remove unbound IgAs. The pellet was resuspended in 1 ml PBS and 10 μl of bacterial suspension were incubated with 25 μl of fecal IgA from C57BL/6 or P2rx7+/− mice at 4°C for 1 h. After two washes, bacteria were incubated for 30 min with biotinylated anti-mouse IgA mAb followed by SYTO-BC and Alexa Fluor 405-labeled streptavidin. The samples were resuspended in 2% paraformaldehyde in PBS for acquisition on a FACSCanto using FSC and SSC parameters in logarithmic mode. To determine the titer of lactobacilli specific IgA in faecal samples, L. reuteri and L. murinus were resuspended at
a density of 10^7 bacteria ml^{-1}. Fresh faecal samples were collected and carefully resuspended in PBS (0.01 g/ml). The obtained suspension was centrifuged two times at 20,000 × g and the supernatant collected to determine the titer of IgA specific for lactobacilli. Faecal samples were serially diluted and 25 μl of each dilution were incubated with 25 μl of bacterial targets suspension at 4 °C for 1 h. After two washes, bacteria were incubated for 30 min with monoclonal FITC anti-mouse IgA and then resuspended in 2% paraformaldehyde in PBS for acquisition on a FACSCanto using FSC and SSC parameters in logarithmic mode. ELISA was used to determine the total IgA concentration in an undiluted aliquot of the same faecal sample used for analysis in flow cytometry. Median fluorescence intensities (MFI) were plotted against IgA concentrations for each sample and 4-parameter logistic curves fitted using Prism (Graphpad, La Jolla, CA). Titters were calculated from these curves as the inverse of the antibody concentration giving an above-background signal. The concentration of total IgA titer required to achieve a given MFI (for example 200) was calculated by re-arrangement of the fitted 4-parameter logistic equation for each sample. As this value is low where a strong antibody response is present, the inverse of this value was plotted. Thus, titers are calculated as the inverse total antibody concentration required to achieve a given MFI. The y-axis value chosen as “above background” necessarily varies between experiments due to the flow cytometer settings, but is constant within any one analysis.

16S rRNA gene sequencing and data analysis. DNA was extracted using the ZR faecal DNA Miniprep kit (Zymo Research, Irvine, CA, USA) following manufacturer's instructions. A primer set specific for the V5–V6 hypervariable regions was used for the amplification of the bacterial 16S rRNA gene (Fw: 5′-ATTAGTACCCCGTATGCG-3′ and Rev: 5′-ACGAGCTGACGACARCCATG-3′)40. The 16S rRNA gene amplicons were then purified and pair-end sequenced on an Illumina MiSeq platform as previously described41. Illumina sequencing resulted in a total of 5,457,629 high quality reads with a mean of 181,921 ± 35,928 sequences per sample. The raw fastq files were submitted to the European Nucleotide Archive with accession number PRJEB20647 (http://www.ebi.ac.uk/ena/data/view/PRJEB20647). Sample accession IDs and metadata, un rarified OTU table and taxonomic classifications are available in the Table S1. Reads were pre-processed using the MICCA pipeline (v1.5.0) (http://www.micca.org)40. The overlapping 2 × 250 paired-end reads were merged using micca mergepairs40. Forward and reverse primer trimming and quality filtering were performed using micca trim and micca filter, respectively. De novo greedy clustering and chimera filtering were performed by using micca otu: operational taxonomic units (OTUs) were assigned by clustering the sequences with a threshold of 97% pairwise identity, and their representative sequences were taxonomically classified using micca classify with the RDP classifier version 2.1142. Singleton OTUs and OTUs present only in the sorted IgA+ and IgA− fractions but not in the pre-sorted faecal samples were discarded from the final OTU table. Multiple sequence alignment was performed using the Nearest Alignment Space Termination (NAST)43 algorithm implemented in micca msa with the template alignment clustered at 97% similarity of the Greengenes database44 (release 13_05). The phylogenetic tree was inferred using micca tree45. Sampling heterogeneity was reduced rarefying samples at the depth of the less abundant sample (56,444 sequences). Alpha- (within-sample richness) and beta-diversity (between-sample dissimilarity) estimates were computed using the phyloseq R package46. Permutational MANOVA (PERMANOVA) was performed on the unweighted UniFrac distance and Bray-Curtis dissimilarity using the adonis() function of the vegan R package with 999 permutations. The identification of taxa differentially distributed in the groups of study was obtained by using the linear discriminant effect size analysis (LEfSe)47. LEfSe ranks features by effect size, meaning the difference in the abundance of taxa identified with 95% confidence intervals in all groups. LEfSe was performed under the following conditions: α value for the statistical test equal to 0.05 and threshold on the logarithmic LDA score for discriminative feature equal to 2.0. Spearman’s correlation coefficients were computed using the psych R package48. All statistical analyses were performed using R49 and GraphPad Prism v7.04 (GraphPad Software, La Jolla, CA, USA). A p-value < 0.05 was considered significant in all cases.

Quantitative PCR of intestinal lactobacilli. Quantification of Lactobacillus in faeces, small intestine and caecal contents was achieved by using the Fast SYBR™ Green Master Mix (Applied Biosystems®™, Waltham, MA, USA) with the QuantStudio 3 Real-Time PCR System (Applied Biosystems®™, Waltham, MA, USA). The PCR reaction mix contained 1X Fast SYBR™ Green Master Mix, 0.4 μM of each Lactobacillus specific primer (F_allact_IS: TGG ATG CCT TGG CAC TAG GA; R_allact_IS: AAA TCT CCG GAT CAA AGC TTA CTT AT)50 and 20 ng of gDNA as template. A seven point standard curve consisting in tenfold serial dilutions of gDNA extracted from a Lactobacillus pure culture at known concentration was used for absolute quantification. Amplification specificity was checked by melting curve analysis, efficiency and reliability of PCR amplifications were also calculated.

Isolation of intestinal Lactobacillus spp. and bacterial cultures. Fresh faeces, small intestinal and caecal contents were collected from P2rx7−/− mice and resuspended 1:10 (weight:volume) in PBS + 0.1% L-cysteine–HCl. The suspensions have been then mixed and tenfold serially diluted. The dilutions were plated on LAMVB medium51 and incubated at 37 °C under anaerobic conditions (AnaeroGen, Oxoid) in jars (AnaeroJar, Oxoid) for 72 h. Based on the identification of different colony morphotypes, 72 isolates have been picked, re-isolated on LAMV AB medium in order to obtain pure colonies and identified by Sanger sequencing of the 16S rRNA gene (8F: AGA GTT TGA TCC TGG CTG AG; 1391R: GAC GGG CCG TGT TCA TAA GT)49. The Lactobacillus isolates were grown in Lactobacillus-MRS broth (EMD Millipore, Burlington, MA, USA) at 37 °C under anaerobic conditions. E. coli DH10B was grown aerobically in Luria–Bertani broth (Sigma-Aldrich, Saint Louis, MO, USA) at 37 °C.
Accession codes. Raw sequences are available in the European Nucleotide Archive (ENA) with Accession Number PRJEB20647 (http://www.ebi.ac.uk/ena/data/view/PRJEB20647). Sample metadata, unrefeared OTU table and taxonomic classifications are available in the Table S1.

References
1. Sommer, F. & Backhed, F. The gut microbiota—masters of host development and physiology. *Nature reviews. Microbiology* **11**, 227–238, https://doi.org/10.1038/nrmicro2974 (2013).
2. Palmer, C., Bbk, E. M., DiGiulio, D. B., Relman, D. A. & Brown, P. O. Development of the human infant intestinal microbiota. *PLoS biology* **5**, e177, https://doi.org/10.1371/journal.pbio.0050177 (2007).
3. Bouskra, D. et al. Lymphoid tissue genesis induced by commensals through NOD1 regulates intestinal homeostasis. *Nature* **456**, 507–510, https://doi.org/10.1038/nature07450 (2008).
4. Fagarasan, S., Kawamoto, S., Kanagawa, O. & Suzuki, K. Adaptive immune regulation in the gut: T-cell-dependent and T-cell-independent IgA synthesis. *Annual review of immunology* **31**, 243–273, https://doi.org/10.1146/annurev-immunol-030409-101314 (2010).
5. Fadlallah, J. et al. Gut microbiota utilize immunoglobulin A for mucosal colonization. *Science* **360**, 795–800, https://doi.org/10.1126/science.aao926 (2018).
6. Nakajima, A. et al. IgA regulates the composition and metabolic function of gut microbiota by promoting symbiosis between bacteria. *The journal of experimental medicine*, https://doi.org/10.1084/jem.20180427 (2018).
7. Bouskra, D. et al. Lymphoid tissue genesis induced by commensals through NOD1 regulates intestinal homeostasis. *Nature* **456**, 507–510, https://doi.org/10.1038/nature07450 (2008).
8. Fagarasan, S., Kawamoto, S., Kanagawa, O. & Suzuki, K. Adaptive immune regulation in the gut: T-cell-dependent and T-cell-independent IgA synthesis. *Annual review of immunology* **31**, 243–273, https://doi.org/10.1146/annurev-immunol-030409-101314 (2010).
9. Nakajima, A. et al. IgA regulates the composition and metabolic function of gut microbiota by promoting symbiosis between bacteria. *The journal of experimental medicine*, https://doi.org/10.1084/jem.20180427 (2018).
10. Bunker, J. F. et al. Innate and Adaptive Humoral Responses Coact Distinct Commensal Bacteria with Immunoglobulin A. *Immunity* **43**, 541–553, https://doi.org/10.1016/j.immuni.2015.08.007 (2015).
11. Kawamoto, S. et al. Foxp3+ T cells regulate immunoglobulin A selection and facilitate diversification of bacterial species responsible for immune homeostasis. *Immunity* **43**, 152–165, https://doi.org/10.1016/j.immuni.2014.05.016 (2014).
12. Khakh, B. S. & North, R. A. P2X receptors as cell-surface ATP sensors in health and disease. *Nature* **442**, 527–532, https://doi.org/10.1038/nature04886 (2006).
13. Choi, Y. S. et al. Bele expressing follicular helper CD4 T cells are fate committed early and have the capacity to form memory. *Journal of immunology* **190**, 4014–4026, https://doi.org/10.4049/jimmunol.1202963 (2013).
14. Gavin, M. A. et al. Foxp3-dependent programme of regulatory T-cell differentiation. *Nature* **445**, 771–775, https://doi.org/10.1038/nature05543 (2007).
15. Proietti, M. et al. ATP-gated ionotropic P2X7 receptor controls follicular helper cell numbers in Peyers’s patches to promote host-microbiota mutualism. *Immunity* **41**, 789–801, https://doi.org/10.1016/j.immuni.2014.10.010 (2014).
16. Perruzza, L. et al. T Follicular Helper Cells Promote a Beneficial Gut Ecosystem for Host Metabolic Homeostasis by Sensing Microbiota-Derived Extracellular ATP. *Cell reports* **18**, 2566–2575, https://doi.org/10.1016/j.celrep.2017.02.061 (2017).
17. Turnbaugh, P. J. et al. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* **444**, 1027–1031, https://doi.org/10.1038/nature05414 (2006).
18. Caesar, R. et al. Gut-derived lipopolysaccharide augments adipose macrophage accumulation but is not essential for impaired glucose or insulin tolerance in mice. *Gut* **61**, 1701–1707, https://doi.org/10.1136/gutjnl-2011-301689 (2012).
19. Todd, J. N. et al. Variation in glucose homeostasis traits associated with P2RX7 polymorphisms in mice and humans. *The Journal of clinical endocrinology and metabolism* **100**, E688–696, https://doi.org/10.1210/jc.2014-4160 (2015).
20. Beaucage, K. L. et al. Loss of P2X7 nucleotide receptor function leads to abnormal fat distribution in mice. *Purinergic Signal* **10**, 291–304, https://doi.org/10.1007/s12070-013-9388-x (2014).
21. Ley, R. E. et al. Obesity alters gut microbial ecology. *Proceedings of the National Academy of Sciences of the United States of America* **102**, 11070–11075, https://doi.org/10.1073/pnas.1004978102 (2005).
22. Turnbaugh, P. J. et al. A core gut microbiome in obese and lean twins. *Nature* **457**, 480–484, https://doi.org/10.1038/nature07540 (2009).
23. Donaldson, G. P., Lee, S. M. & Mazmanian, S. K. Gut microbiota in human disease. *Nature reviews. Microbiology* **14**, 20–32, https://doi.org/10.1038/nrmicro3552 (2016).
24. Caesar, R., Tremaroli, V., Kovatcheva-Datchary, P., Cani, P. D. & Backhed, F. Crosstalk between Gut Microbiota and Dietary Lipids Aggravates WAT Inflammation through TLR Signaling. *Cell Metab* **22**, 658–668, https://doi.org/10.1016/j.cmet.2015.07.026 (2015).
25. Furet, J. P. et al. Differential adaptation of human gut microbiota to bariatric surgery-induced weight loss: links with metabolic and low-grade inflammation markers. *Diabetes* **59**, 3049–3057, https://doi.org/10.2337/db10-0253 (2010).
26. Ubeda, C. et al. Intestinal microbiota containing *Barnesiella* species cures vancomycin-resistant Enterococcus faecium colonization. *Infection and immunity* **81**, 965–973, https://doi.org/10.1128/IAI.01197-12 (2013).
27. Atarashi, K. et al. Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. *Nature* **500**, 232–236 (2013).
28. Cho, I. et al. Antibiotics in early life alter murine colonic microbiome and adiposity. *Nature* **488**, 621–626, https://doi.org/10.1038/nature11400 (2012).
29. McBride, K. et al. The gut microbiota is required for normal development of thymic T cells in the absence of conventional T cells. *Journal of immunology* **188**, 6827–6835, https://doi.org/10.4049/jimmunol.1302707 (2017).
30. Million, M. et al. The gut microbiota regulates the development of the human infant intestinal microbiota. *Nature* **455**, 104–107, https://doi.org/10.1038/nature07278 (2008).
31. Shih, S. et al. Preparing the gut with antibiotics enhances gut microbiota reprogramming efficiency by promoting xenomicrobiota colonization. *Frontiers in microbiology* **8**, 1208, https://doi.org/10.3389/fmicb.2017.01208 (2017).
32. Moor, K. et al. Analysis of bacterial-surface-specific antibodies in body fluids using bacterial flow cytometry. *Nat Protoc* **11**, 1531–1533, https://doi.org/10.1038/nprot.2016.091 (2016).
51. Hartemink, R., Domenech, V. & Rombouts, F. LAMV AB—a new selective medium for the isolation of lactobacilli from faeces.

50. Haarman, M. & Knol, J. Quantitative real-time PCR analysis of fecal bacteria.

49. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, 2013.

48. Revelle, W. Psych: procedures for psychological, psychometric, and personality research. R package version 1.3-10. Northwestern University, Evanston, IL (2013).

47. Segata, N. Metagenomic biomarker discovery and explanation. Genome biology 12, R60, https://doi.org/10.1186/gb-2011-12-6-r60 (2011).

46. McMurdie, P. J. & Holmes, S. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PloS one 8, e61217, https://doi.org/10.1371/journal.pone.0061217 (2013).

45. Price, M. N., Dehal, P. S. & Arkin, A. P. FastTree 2: approximately maximum-likelihood trees for large alignments. PloS one 5, e9490, https://doi.org/10.1371/journal.pone.009490 (2010).

44. DeSantis, T. Z. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Applied and environmental microbiology 72, 5069–5072, https://doi.org/10.1128/AEM.03006-05 (2006).

43. DeSantis, T. Z., et al. NAST: a multiple sequence alignment server for comparative analysis of 16S rRNA genes. Nucleic acids research 34, W398-W399 (2006).

42. De Filippis, Claudia Lionetti (University of Bari), and Caterina Manzari (Institute of Biomembranes and Bioenergetics) for contributing to 16S rRNA gene sequencing. This research was supported by the Swiss National Science Foundation (Grants 310030_159491 and IZCNZ0-174704 to F.G.) and Novartis Stiftung für medizinisch-biologische Forschung (grant 18B096 to F.G.).

Acknowledgements
We would like to thank David Jarrossay (Institute for Research in Biomedicine) for cell sorting and Teresa De Filippis, Claudia Lionetti (University of Bari), and Caterina Manzari (Institute of Biomembranes and Bioenergetics) for contributing to 16S rRNA gene sequencing. This research was supported by the Swiss National Science Foundation (Grants 310030_159491 and IZCNZ0-174704 to F.G.) and Novartis Stiftung für medizinisch-biologische Forschung (grant 18B096 to F.G.).

Author Contributions
L.P., E.S. and F.G. designed the experiments. L.P. and E.S. performed experiments and analysed data. A.M.D. performed the 16S rRNA gene sequencing. G.G., B.F. and S.G. analysed data. F.G. wrote the manuscript. F.G., L.P. and F.S. designed the experiments. L.P. and F.S. performed experiments and analysed data. A.M.D. conceived the study and revised the manuscript. All the authors critically reviewed and approved the manuscript.

Additional Information
Supplementary information accompanies this paper at https://doi.org/10.1038/s41598-019-45724-9.

Competing Interests: The authors declare no competing interests.

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2019