Distinct B Cell Subsets in Peyer's Patches Convey Probiotic Effects by Lactobacillus Reuteri

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Research

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Abstract

Background: Intestinal Peyer's patches (PPs) form unique niches for bacteria-immune cell interactions that direct host immunity and shape the microbiome. Here we investigate how peroral administration of probiotic bacterium Lactobacillus reuteri R2LC affects B lymphocytes and IgA induction in the PPs, as well as the downstream consequences on 28 intestinal microbiota and inflammation susceptibility.

Results: The B cells of PPs were separated by size to circumvent activation-dependent cell identification biases due to dynamic expression of markers, which resulted in two phenotypically, transcriptionally and spatially distinct subsets: small IgD+/GL7- /S1PR1+/Bcl6, CCR6-expressing pre-germinal center (GC)-like B cells with innate-like functions located subepithelially, and large GL7+/S1PR1-/Ki67+/Bcl6, CD69-expressing B cells with strong metabolic activity found in the GC. Peroral L. reuteri administration expanded both B cell subsets, and enhanced the innate-like properties of pre-GC-like B cells while retaining them in the sub-epithelial compartment by increased sphingosine-1- phosphate/S1PR1 signaling. Furthermore, L. reuteri promoted GC-like B cell differentiation, which involved expansion of the GC area and autocrine TGFβ-1 activation. Consequently, PD-1-T follicular helper cell-dependent IgA induction and production was increased by L. reuteri, which shifted the intestinal microbiome and protected against dextran-sulfate-sodium induced colitis and dysbiosis.

Conclusions: The Peyer's patches sense, enhance and transmit probiotic signals by increasing the numbers and effector functions of distinct B cell subsets, resulting in increased IgA production, altered intestinal microbiota and protection against inflammation.

Full Text

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Figures

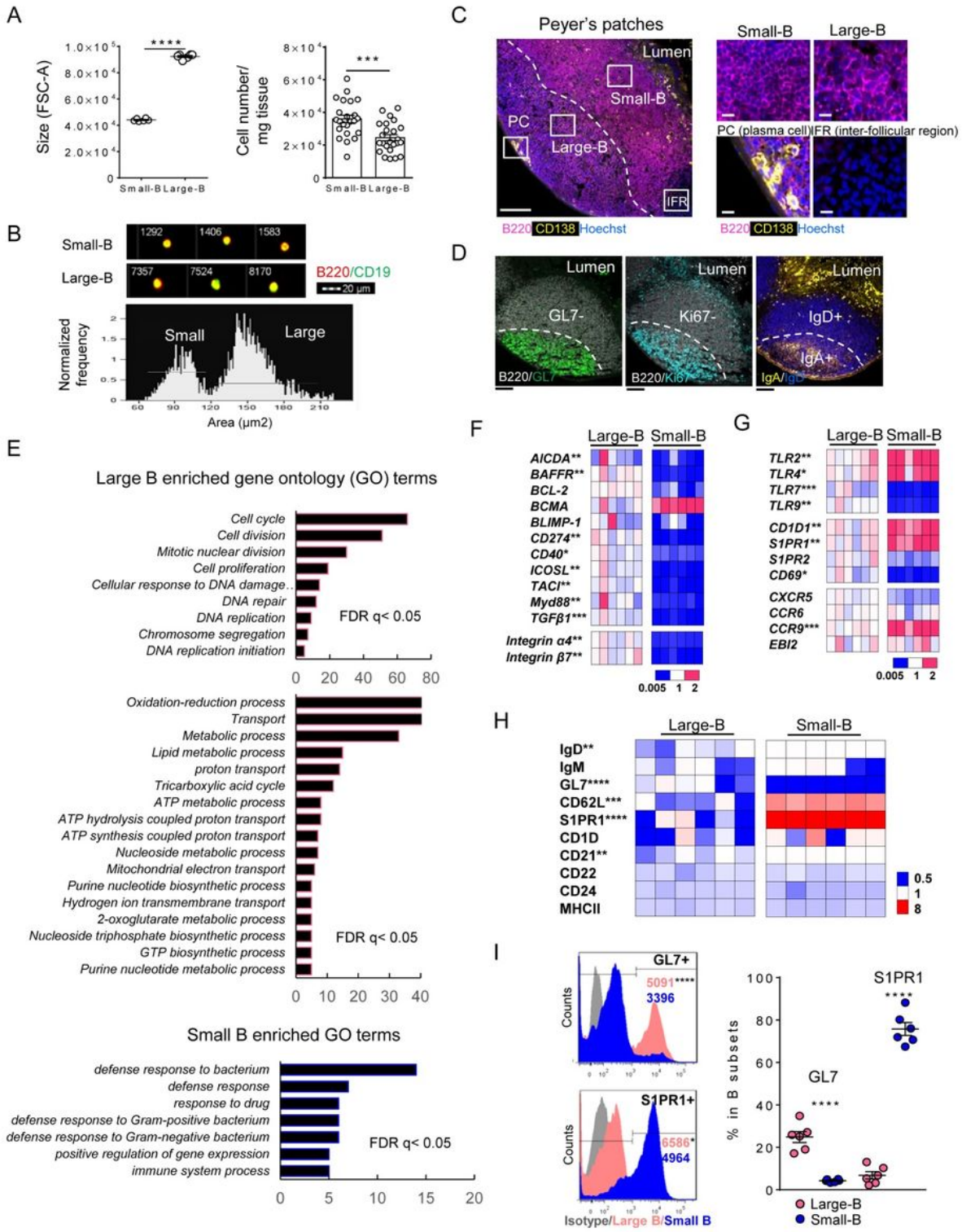

Figure 1

Phenotypic and transcriptional distinction between B cell subsets in Peyer's patches. a Flow cytometry of PPs live CD3-CD19+B220+ B lymphocytes separating large B cells from small B cells by forward scatter (FSC-A, n = 6), and the number of small B and large B cells per mg tissue (n = 24). b Imaging flow cytometry of the areas (μm2) of small B and large B cell from at least three independent experiments. c, d Immunohistochemistry of PPs stained with anti-B220 (magenta), anti-CD138 (yellow) and Hoechst (blue)

in C or anti-B220 (white), anti-GL7 (green), anti-Ki67 (cyan), anti-IgA (yellow) and anti-IgD (blue) in (d). Scale Bars equal 100 μm or 10μm in closed-up reviews. e Enrichment of gene ontology categories (Biological Process, BP) for genes differentially expressed in large vs. small B cells determined by microarray of lin-CD19+B220+ cells sorted on FSC-A (n = 4), the number of genes in each functional category is shown. Data were adjusted by false discovery rate control (FDR). f, g Displays of heat maps of genes expression by q-RT-PCR in sorted small and large B cells. The expression was normalized to the mean value of large B and each column represents one sample. h, i Heat maps and histograms depicting expression of surface markers on small and large B cells (n = 6). The frequency of indicated markers (normalized to the mean value of large B cells). i MFI of B cells positive for GL7 and S1PR1, and percentage in each subset. Data are presented as mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ using two tailed Student's t test.

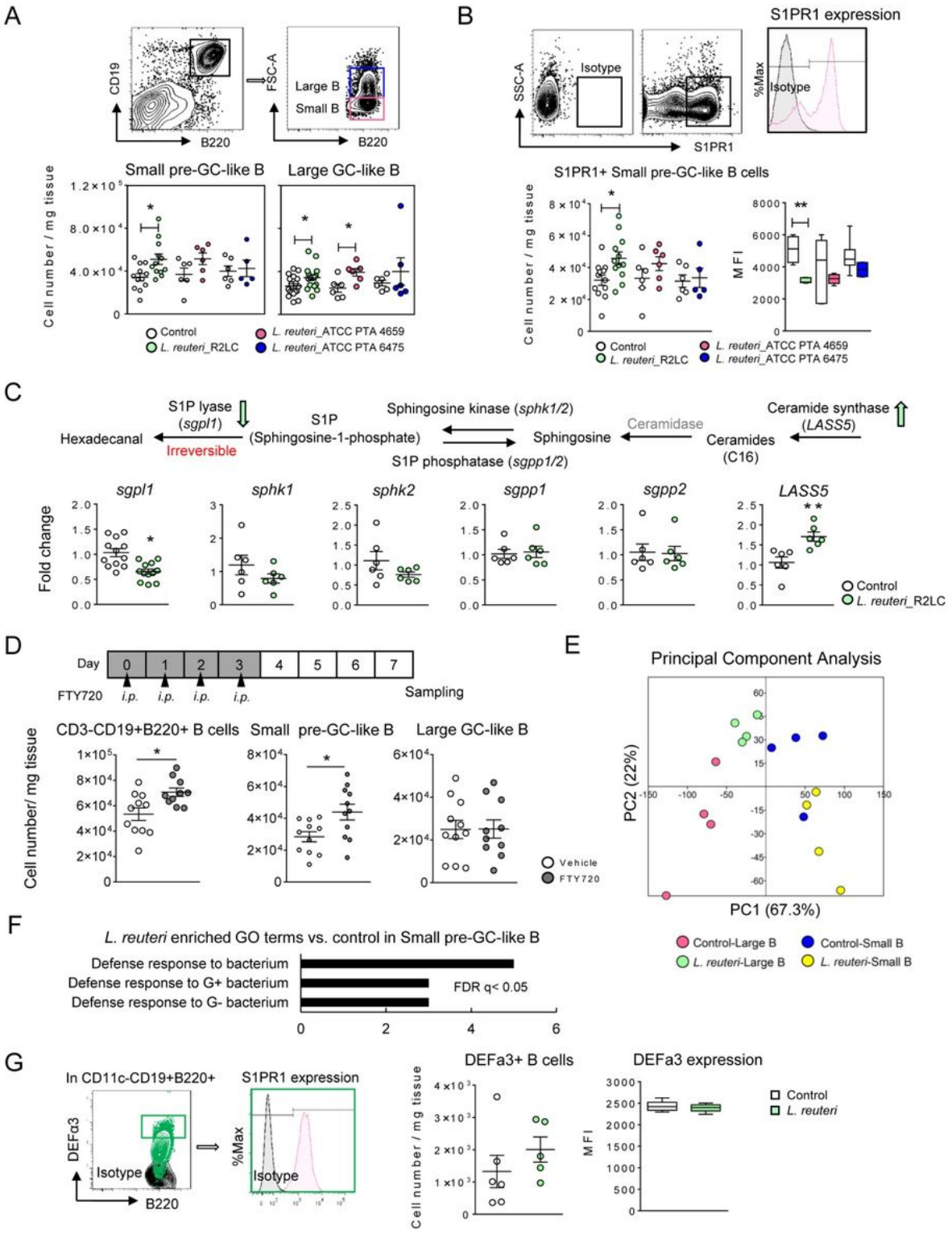

Figure 2

Lactobacillus reuteri increases the population of Peyer's patches B cells and enhances their effector functions. a Flow cytometry of small-pre-GC-like and large-GC-like B cell subsets from control mice or mice treated with 108 Lactobacillus reuteri strains (R2LC, 4659 or 6475) perorally for 7 consecutive days (n = 5-12). b Numbers of S1PR1+ small pre-GC-like B cells (CD3-CD19+B220+) and their S1PR1 expression (MFI, n = 5-12). c Illustration of enzymes regulating sphingosine-1-phosphate (S1P)

homeostasis (top). q-RT-PCR analysis of gene expression (fold change, n = 6) of the S1P pathway (bottom). d Experimental design evaluating the effect of the sphingosine-1-phosphate (S1P) receptor modulator FTY720 (top), and flow cytometry of B cell subsets (lower panels, n = 10-11). e Principal component analysis of the microarray data, where each dot represents one sample (n = 4). f Enrichment of gene ontology categories (BP) for genes upregulated by L. reuteri in small B cells. g Flow cytometry of DEFa3+ B cells (CD11C-CD19+B220+) in PPs and DEFa3 expression (MFI, n = 5-6). Data are presented as mean ± SEM. *p < 0.05, **p < 0.01 using two tailed Student's t test.

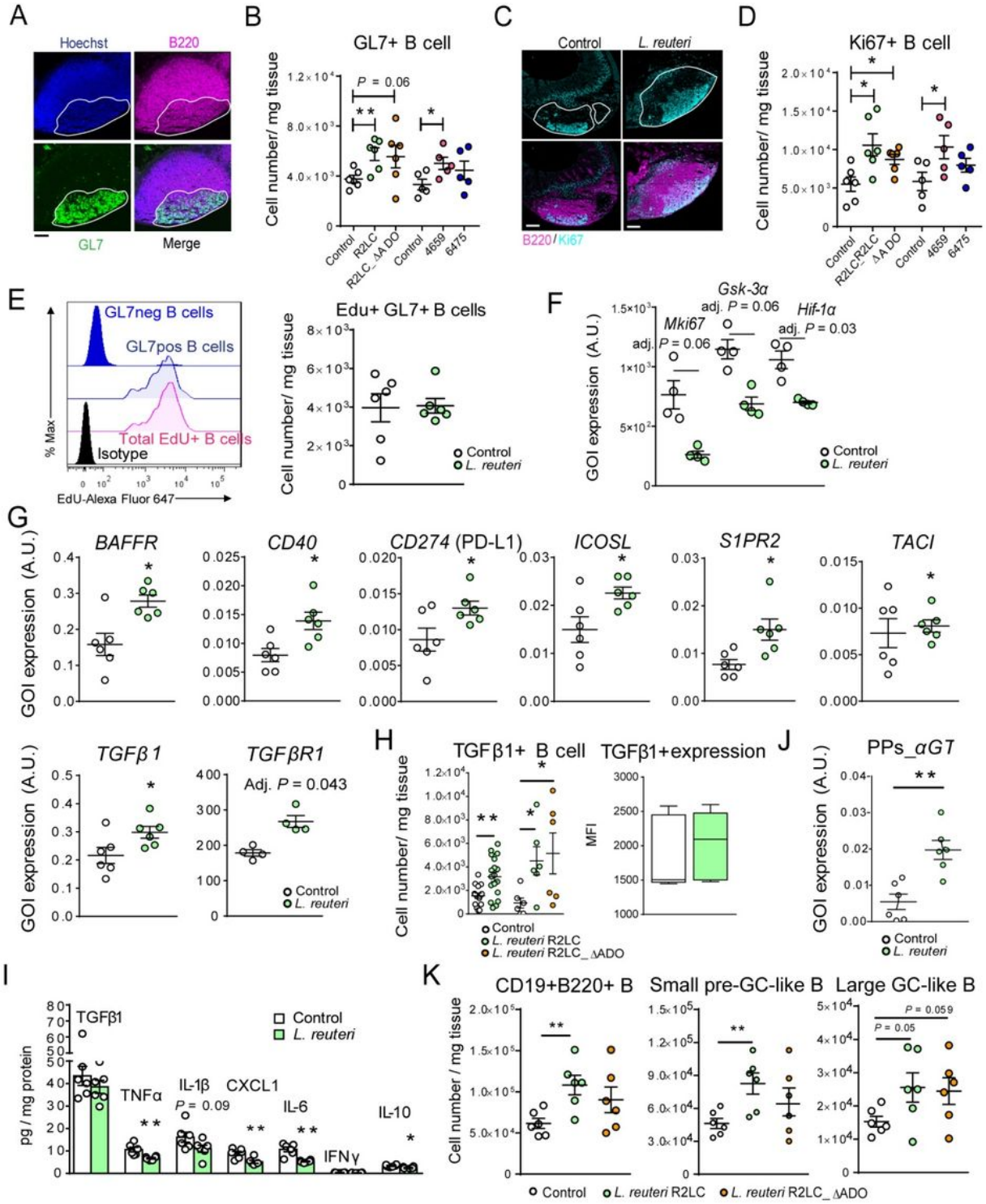

Figure 3

L. reuteri expands the GC-like B cell population and reprograms its functional gene signature. a, c Immunohistochemistry of the GL7+ area stained with anti-B220 (magenta), anti-GL7 (green) and Hoechst (blue), and Ki67+ cells in PPs stained with anti-Ki67 (cyan, n = 8-11). Scale bars equal 100 μm. Representative images are shown in a and c, and the corresponding quantification shown in Fig. S3F (n = 8-9) and Fig. S3G (n = 8-9). b, d Flow cytometry quantification of GL7+ B cells (in CD3-CD19+B220+, n = 5-6) and Ki67+ cells (n = 5-6). e In vivo proliferation assay was performed by flow cytometry following i.p. injection of EdU. A histogram depicting EdU expression in GL7-, GL7+ or EdU+CD19+B220+B cells and the number of EdU+GL7+ B cells (n = 6). f Expression of Mki67, Gsk-3α and Hif-1α in large GC-like B cells from control or L. reuteri-treated mice (GOI, gene of interest; A.U., arbitrary unit). g q-RT-PCR analysis of gene expression levels (n = 6) and expression of TGFβR1 (microarray analysis). h Flow cytometry analyzing the numbers of TGFβ1+ B cells as well as the TGFβ1 levels (MFI, n = 6-18). i The PPs tissue was analyzed for cytokine/chemokine production by Multi-Plex Mesoscale and ELISA, normalized to tissue protein content (n = 6). j Expression of α germline transcripts (αGT) in PPs (n = 6). k The numbers of B cells of different subsets in PPs in untreated mice and in response to L. reuteri R2LC and L. reuteri R2LC_ΔADO (n = 6). Data are presented as mean ± SEM. *$p < 0.05$, **$p < 0.01$ using Student's t test or ANOVA with Tukey's post hoc test.

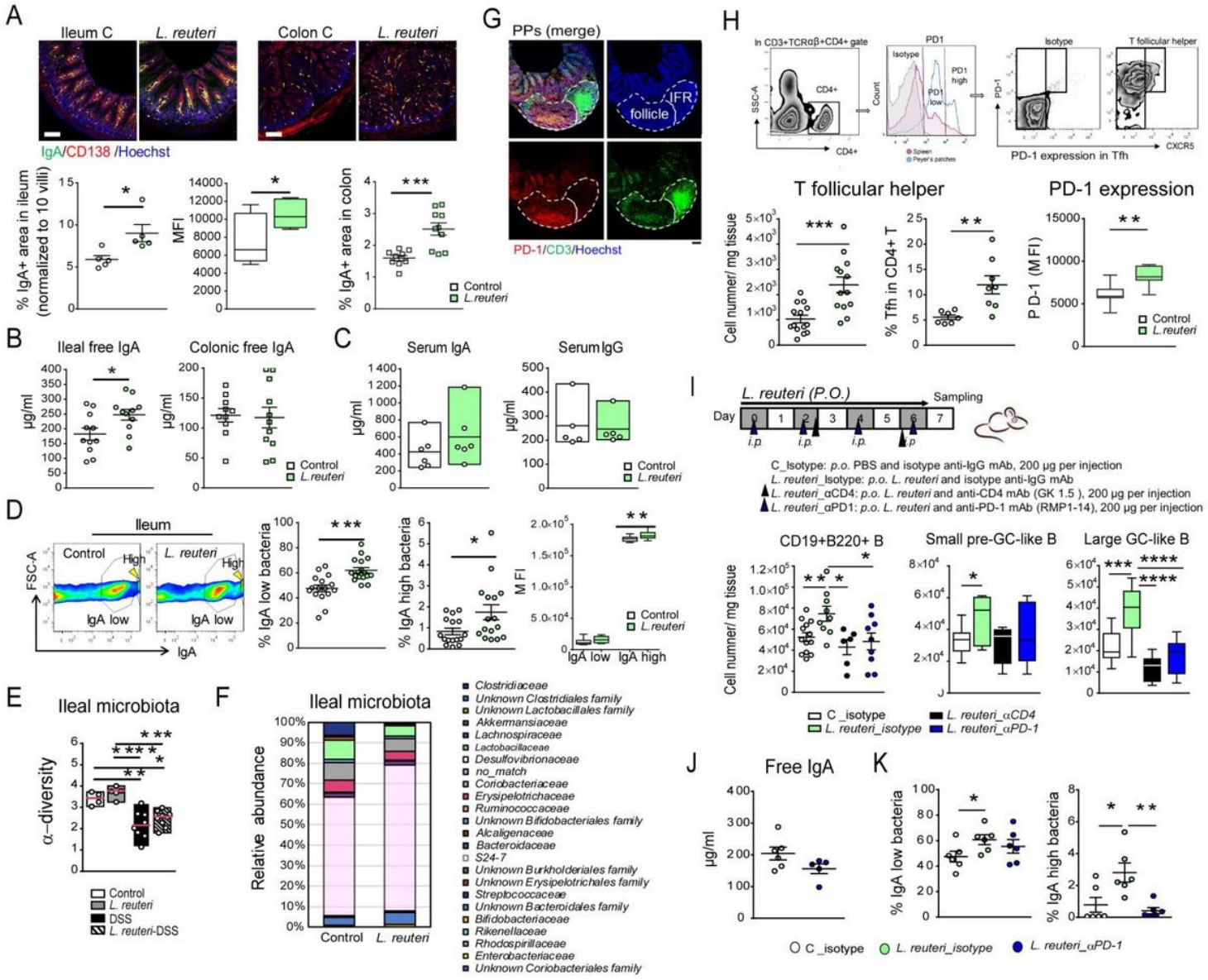

Figure 4

L. reuteri promotes B cell IgA-responses in a Tfh-PD-1-dependent manner. a The area of IgA+ plasma cells (anti-CD138) quantified in ileal and colonic tissues, n = 5 (duplicate/per mouse, scale bars equal 100 µm). b Free IgA (µg/ml, ELISA) in the lumen of ileum and colon (n = 10-11) and c Serum IgA and IgG concentrations (µg/ml, ELISA, n = 5-6). d Flow cytometry of IgA+ bacteria in ileum (n = 16-17). e, f Ileal microbiome assessed by 16S rRNA gene amplicon sequencing. Microbial community diversity was calculated as α-diversity in mice treated with L. reuteri and/or DSS compared to controls, n = 4-9. Data are presented as median values. g Localization of PD-1+ T cells in PPs stained with anti-PD-1 (red), anti-CD3 (green) and Hoechst (blue, scale bar equals 100 µm) from three independent experiments. h Flow cytometry of Tfh cells and PD-1 expression (n = 13-14). A histogram of PPs and spleen indicating a uniquely high expression of PD-1 in PPs T cells. i Design of PD-1 monoclonal antibodies blocking (blue arrows) and CD4+ T cell depletion (black arrows) experiments and effects on the number of B cell subsets (n = 6-13). j, k Free IgA production and quantification of IgA+ bacteria in the ileum from PD-1

(mAb) blocking experiments (n = 6). Data are presented as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 using two tailed Student's t test or ANOVA with Tukey's post hoc test.

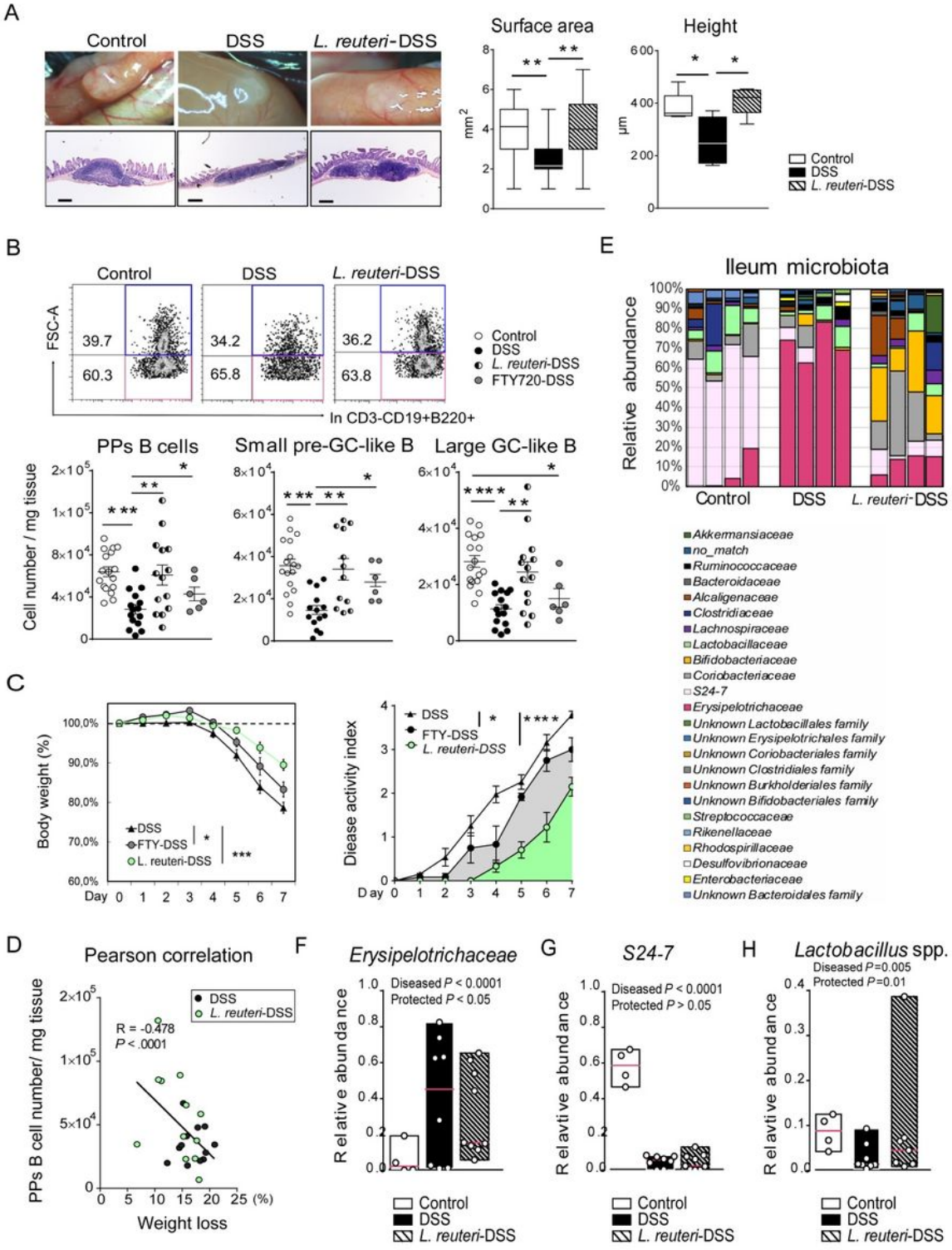

Figure 5

L. reuteri provides protection against DSS-induced colitis, disruption of Peyer's patches and intestinal dysbiosis. a Macroscopic analysis of the PPs surface area (mean area of PPs per mouse, mm2, n = 5) in WT mice (control), WT mice receiving DSS (DSS, 3% in drinking water for 7 consecutive days) as well as

mice receiving L. reuteri R2LC was given daily to WT mice for 14 days starting 7 days prior to DSS-treatment (L. reuteri-DSS). Histological analysis of the PPs height of H&E-stained serial sections (μm, n = 5, scale bars equal 200 μm). b Flow cytometry of 831 live CD3-CD19+B220+ B cells, small-pre-GC-like and large-GC-like B cells in PPs (n = 6-17). c Body weight change and disease activity index of DSS-treated mice and mice co-treated with L. reuteri (n = 9) or FTY720 (n = 6). d Pearson correlation of PPs B cell number and body weight loss (%) (n = 24). e Bar graph depicts bacterial community composition of individual mouse, n = 4. f, g, h Relative abundance of Erysipelotrichaceae, S24-7 and Lactobacillus spp. in the ileal microbiota (n = 4-9). Data are presented as median values. Contrast analysis demonstrated that bacterial taxa changed significantly with DSS-treatment (Diseased $P < 0.05$) and was preserved by L. reuteri-treatment (Protected $P < 0.05$). Data are presented as mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ using ANOVA with Tukey's post hoc test.

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