Supporting Information

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SI Methods

Intragastric Sensitization with Peanut/Cholera Toxin. In all experiments, specific pathogen-free mice were sensitized by intragastric gavage with 6 mg of peanut (PN) plus 10 μg cholera toxin (CT, List Biologicals) on days 0 and 2 and with 6 mg of PN plus 15 μg CT on days 7, 14, 21, and 28. The dose of CT was reduced to 10 μg for each sensitization of gnotobiotic mice. Control groups received CT in Tris buffer as a vehicle. When gnotobiotic or Abx-treated mice were colonized, sensitization began 1 d after fecal gavage. On day 35, all mice were challenged twice by gavage with 20 mg of PN 30 min apart. Rectal temperature was recorded before and 30 min after each challenge; the reported ΔT is the temperature 30 min after the second challenge minus the temperature prechallenge. Serum, feces, and tissues were harvested from each mouse on day 36.

Bacterial Colonization. For *Bacteroides* isolation, full-length 16S rDNA was PCR amplified and sequenced from individual colonies from SPF feces after growth on Anaerobic Neomycin Blood Agar plates (Remel). A strain with >99% sequence identity to *Bacteroides uniformis* (NR_040866) was further propagated in Schaedler's media. For Clostridia isolation, a chloroform-treated fecal/cecal suspension was prepared with slight modifications from ref. 1. A single fecal pellet plus an equivalent volume of cecal material from the same donor was suspended in 18 mL of prereduced PBS in an anaerobic chamber. Chloroform was added to 3% (vol/vol) concentration; the sample was shaken vigorously and then incubated at 37 °C for 1h. Chloroform was removed by percolation with CO_2 from a compressed cylinder. This suspension was then administered to an adult germ-free (GF) mouse as a repository for all subsequent colonizations, which were performed as described in the main text. Conventionalization of GF or antibiotic (Abx)-treated mice was performed by suspending a fecal pellet and an equivalent volume of cecal material from a young donor SPF mouse in 1.5 mL sterile PBS and administering 100 μL of the liquid phase to weanling C57BL/6 or C57BL/6 Foxp3^{gfp} recipients by gavage. A second gavage was given 2 wk after weaning in Abx conventionalized and Abx Clostridia sensitization experiments. For Abx recovery experiments, mice received 1 wk of Abx by gavage before weaning and then were left unmanipulated; no additional bacteria were administered. Whenever possible, littermates were used to minimize variation in the composition of the microbiota pretreatment. If littermates were not used, all pups were related by maternal lineage.

Ig Detection. For antigen-specific assays, plates were coated with PN, and antigen-specific serum antibodies were detected with goat anti-mouse IgG1-HRP (Southern Biotech), rat anti-mouse IgE-AP (23G3, Southern Biotech), or goat anti-mouse IgG-HRP (Southern Biotech). PN-specific standards were prepared from the serum of sensitized mice by affinity purification on a PN-conjugated CNBr-Sepharose column (GE Healthcare). For PN-IgE standard, IgG was depleted with Protein G Sepharose (GE Healthcare) before affinity purification. Total IgE plates were coated with purified rat anti-mouse IgE (R35-72, BD Pharmingen), and antibodies were detected with biotin rat anti-mouse IgE (R35-118, BD Pharmingen) and streptavidin-HRP using a mouse IgE standard (BD Biosciences). For fecal IgA, fecal pellets were suspended in PBS containing 0.01% NaN_3 (1 mL/0.1 g feces) and centrifuged to remove debris. Fecal supernatants were applied to plates coated with goat anti-mouse polyclonal IgA (Southern Biotech), and antibodies were detected with goat anti-mouse IgA-AP (Southern Biotech). OD was normalized to a commercial mouse IgA standard (BD Biosciences).

Preparation of 16S rRNA-Based Amplicon Library and Data Analysis. Fecal samples were mixed with 0.1 mm zirconia/silica beads in 1.4 mL ASL buffer (Qiagen) in a Mini-Beadbeater (Biospec); DNA was extracted with the QIAamp DNA Stool Mini Kit (Qiagen) using 1/2 inhibitEX tablet and 100 μL Buffer AE for elution. Fecal DNA template was amplified using the 515F/806R region of the 16S rRNA gene with primers and cycling conditions modified slightly from ref. 2, specifically adapted for the Illumina MiSeq by adding nine extra bases in the adapter region of the forward amplification primer to support paired-end sequencing. Briefly, the V4 region of the 16S rRNA gene was amplified with region-specific primers that included the Illumina flowcell adapter sequences and 12-base barcodes on the reverse primer. PCR reactions were completed in triplicate, and products were pooled. Each pool was then quantified using Invitrogen's PicoGreen, pooled with equal amounts of DNA per sample, and cleaned using the UltraClean PCR Clean-Up Kit (MoBIO). Amplicons were then sequenced on an Illumina MiSeq at the Next-Generation Sequencing Core at Argonne National Laboratory using custom sequencing primers and procedures described in the supplementary methods of ref. 3. Paired-end Illumina reads were joined using fastq-join (E. Aronesty, code.google.com/p/ea-utils). All analysis of high-throughput sequencing data was performed using QIIME v.1.6.0 as previously described (4), except that uclust was used for operational taxonomic unit (OTU) selection (with 97% identity threshold). Where the UniFrac metric was used, principal coordinates analysis of the unweighted pairwise distance matrix is shown; even sampling was performed at a depth of 7,500 (Fig. S2*F*) or 13,500 (Fig. 5*I*) sequences per sample. Phylogenetic reconstruction of Clostridia OTUs (Fig. S1*C*) includes sequences described in ref. 5.

Calculation of Bacterial Load. Bacterial load was determined by quantitative real-time PCR using a protocol modified from ref. 6. Fecal DNA was extracted as for sequencing, and bacterial load was quantified against a standard curve derived from a pCR4TOPO-TA vector containing a nearly full-length copy of the 16S rRNA gene from a member of *Porphyromonadaceae*. Bacterial DNA was amplified with universal primers 8F and 338R using the iQ SYBR green supermix (Bio-Rad Life Science) and the StepOnePlus system (Applied Biosystems). The results were normalized to fecal weight.

Isolation of Lymphocytes and Flow Cytometry. Single cell suspensions from spleen, mesenteric lymph node, and lamina propria (LP) were stained with anti-CD4-PE (RM 4.4, eBioscience). Intracellular staining with anti-Foxp3-FITC (FJK-16s, eBioscience) was performed after permeabilization with Foxp3 Fix/Perm buffer (eBioscience). In experiments with gnotobiotic mice, Tregs were analyzed 2 wk after colonization. For innate lymphoid cell (ILC) cytokine analysis, cells were isolated and stained as described in ref. 7. Briefly, mononuclear cells from the colon were isolated using mechanical/enzymatic digestion and Percoll density centrifugation. The isolated cells were incubated for 4 h with 50 ng/mL phorbol myristate acetate and 500 ng/mL ionomycin before fixation and permeabilization using Foxp3 Fix/Perm buffer (eBioscience) and staining for CD3, CD4, TCRβ, NKp46, RORγt, and IL-22. All samples were acquired on FACSCanto or LSRII, and data were analyzed with FlowJo software (TreeStar).

Microarray Analysis. Data were normalized using Illumina software and then analyzed with dChip. Genes were considered induced if the detection *P* value for each sample was >0.05 and there was ≥1.5-fold increase in expression in either colonized group compared with GF. For verification of results, cDNA was produced as described in *Methods* followed by quantitative real-time PCR using Taqman primer/probe sets and Master Mix (Applied Biosystems). Results were normalized to *Gapdh*.

Mucus Staining and Goblet Cell Quantification. Tissue from the center of the distal colon was collected from GF, *B. uniformis*, and Clostridia-colonized mice 6 d after colonization or from sensitized Abx-treated, Clostridia-colonized mice with or without anti-IL-22 treatment 24 h after challenge. Tissue was fixed for 4 h at 4 °C in Carnoy's fixative and then transferred to 70% EtOH for paraffin embedding. Cross-sections of 5-μm thickness were stained with periodic acid Schiff as previously described (8). Slides were imaged on a CRi Panoramic Scan Whole Slide Scanner and analyzed with Panoramic Viewer. Goblet cells were quantified in at least 7 crypts per mouse, average 11 crypts per mouse.

Cell Culture and Cytokine Measurement. Single-cell suspensions were prepared from spleens harvested 24 h after challenge from Abx-treated, Clostridia-colonized mice treated with anti-IL-22 or an isotype control and sensitized with PN and CT. Cells were plated at 2×10^5 cells per well with media alone, 1 μg/mL anti-CD3 (clone 2C11), or 200 μg/mL PN and incubated at 37 °C for 72 h as previously described (9). After 72 h, plates were frozen at −20 °C. Cytokine concentrations in supernatants were measured using Milliplex MAP Mouse Cytokine magnetic bead panel (Millipore) and read on a Bio-Plex machine (BioRad) as previously described (10).

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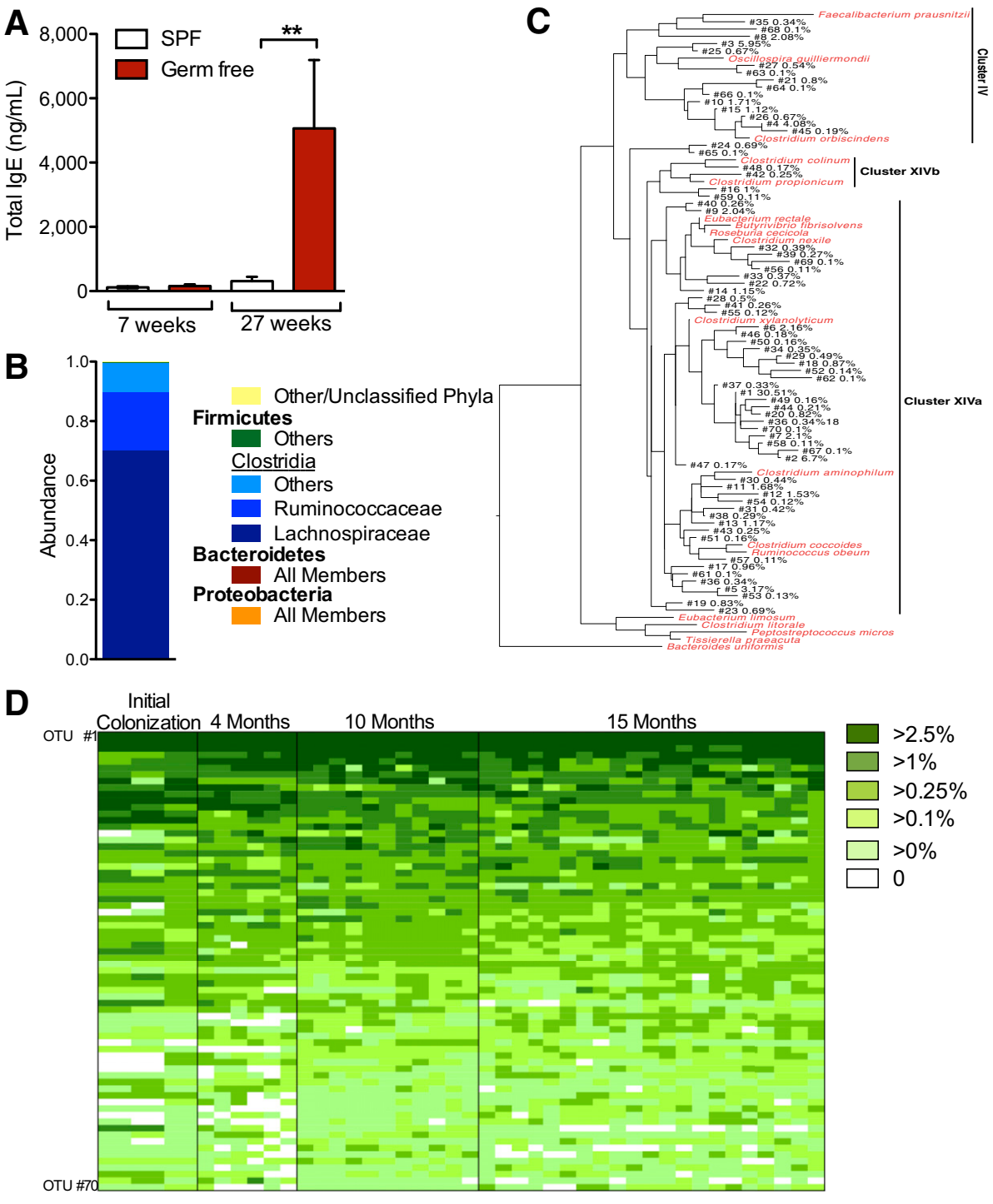

Fig. S1. (*A*) IgE concentrations determined by ELISA. $n = 7–8$ mice per group. (*B*) Average taxonomic classifications from paired-end Illumina sequencing of fecal 16S rDNA amplicons from 41 Clostridia-colonized mice over a 15-mo period. (*C*) Phylogenetic reconstruction of OTUs present in feces from the same mice as in *B*. Among reads classified as Clostridia, all OTUs (defined as 97% identical) with >0.1% abundance were considered for alignment with representative sequences (Red text; pynast). OTUs are numbered 1–70, and their abundance is shown as a percentage. (*D*) Abundance of the 70 OTUs depicted in *C* in individual mice over time. $^{**}P < 0.01$ by one-way ANOVA with Tukey posttest.

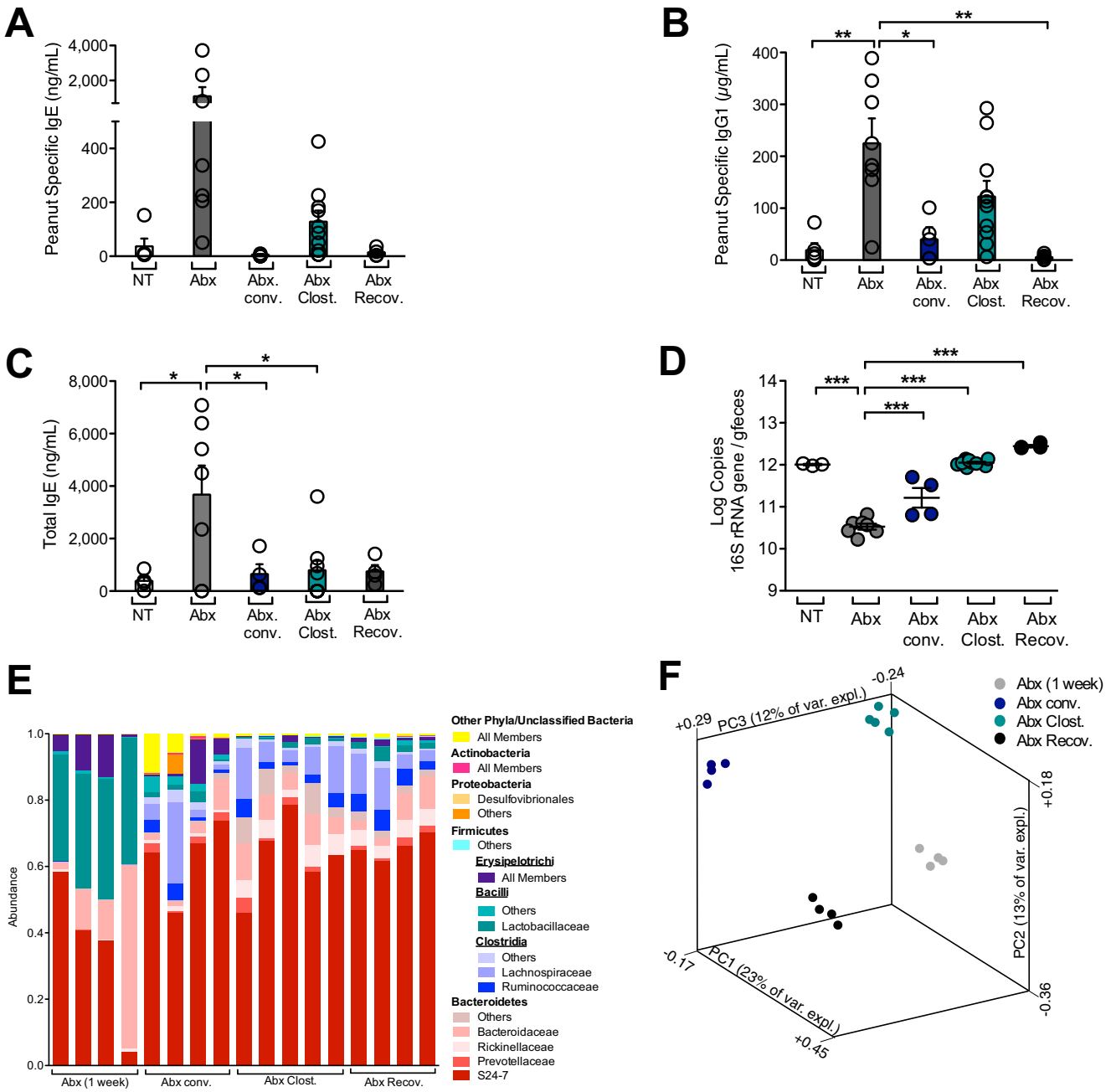

Fig. S2. (*A–D*) Groups of mice were not treated (NT, white) or treated with Abx by gavage before weaning and then in the drinking water afterward for 5 wk (as in Fig. 1; Abx, dark gray). Alternatively, groups of mice received Abx by gavage preweaning and then were colonized at weaning with conventional SPF feces and cecal contents (Abx. conv., blue), or with a consortium of Clostridia (Abx Clost., green), or were allowed to recover without colonization (Abx Recov., black). Mice in each treatment group were sensitized with PN/CT and challenged on day 35. (*A*) Concentration of PN-specific IgE, (*B*) IgG1, and (*C*) total IgE in the serum of sensitized mice, collected 24 h after challenge, as determined by ELISA. $n = 4–10$ mice per group. (*D*) Bacterial load at killing. $n = 3–5$ mice per group. (*E*) Taxonomic classifications and (*F*) UniFrac analysis for Illumina-derived sequences of fecal 16S rDNA from mice in *A–D*. $n = 4–5$ mice per group. Each group was housed in two cages, separated by sex. Data shown are pooled from two independent experiments. Bar graphs depict mean and SEM; in *A–D* and *F*, each circle represents an individual mouse. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ by one-way ANOVA with Tukey posttest (*A–D*).

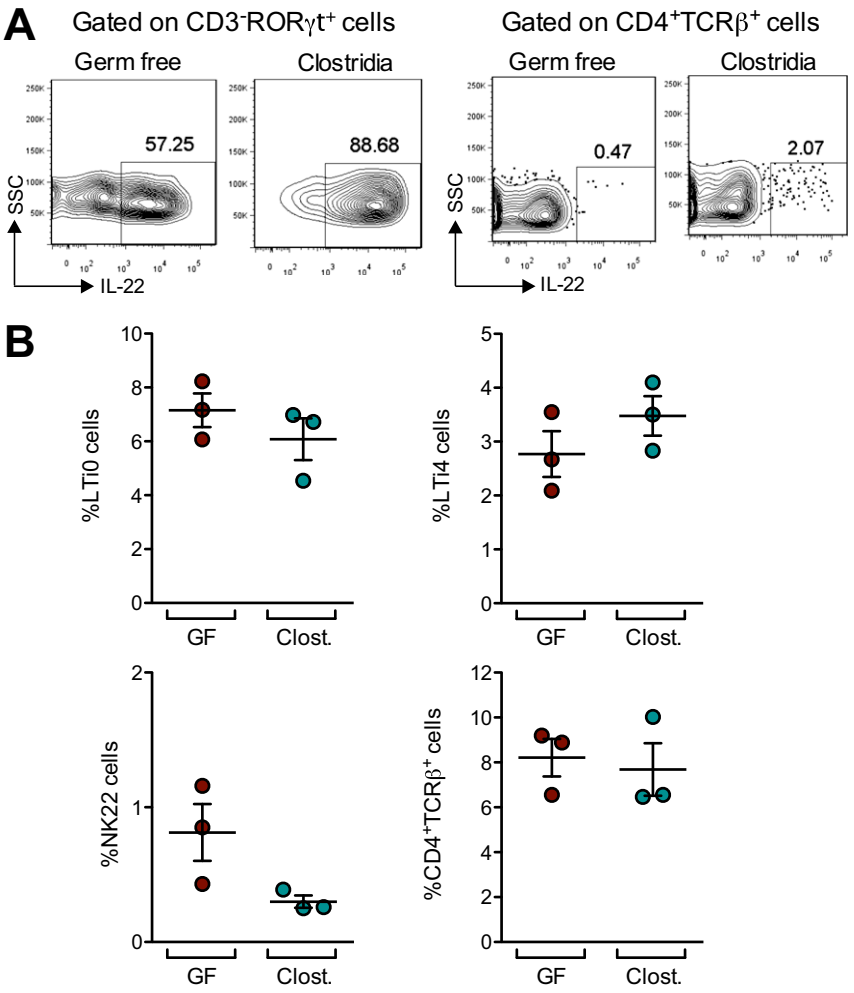

Fig. S3. (*A*) Representative flow cytometry plots for data in Fig. 4*B*. (*B*) Percentage of Lti0 ($CD3^{-}CD4^{-}ROR\gamma t^{+}NKp46^{-}$), Lti4 ($CD3^{-}CD4^{+}ROR\gamma t^{+}NKp46^{-}$), and NK-22 ($CD3^{-}ROR\gamma t^{+}NKp46^{+}$) ILC subsets among $CD3^{-}TCR\beta^{-}$ cells and the percentage of $CD4^{+}TCR\beta^{+}$ cells in the colonic LP of GF (red) and Clostridia-colonized (green) mice. $n = 3$ mice per group. Data are representative of three independent experiments.

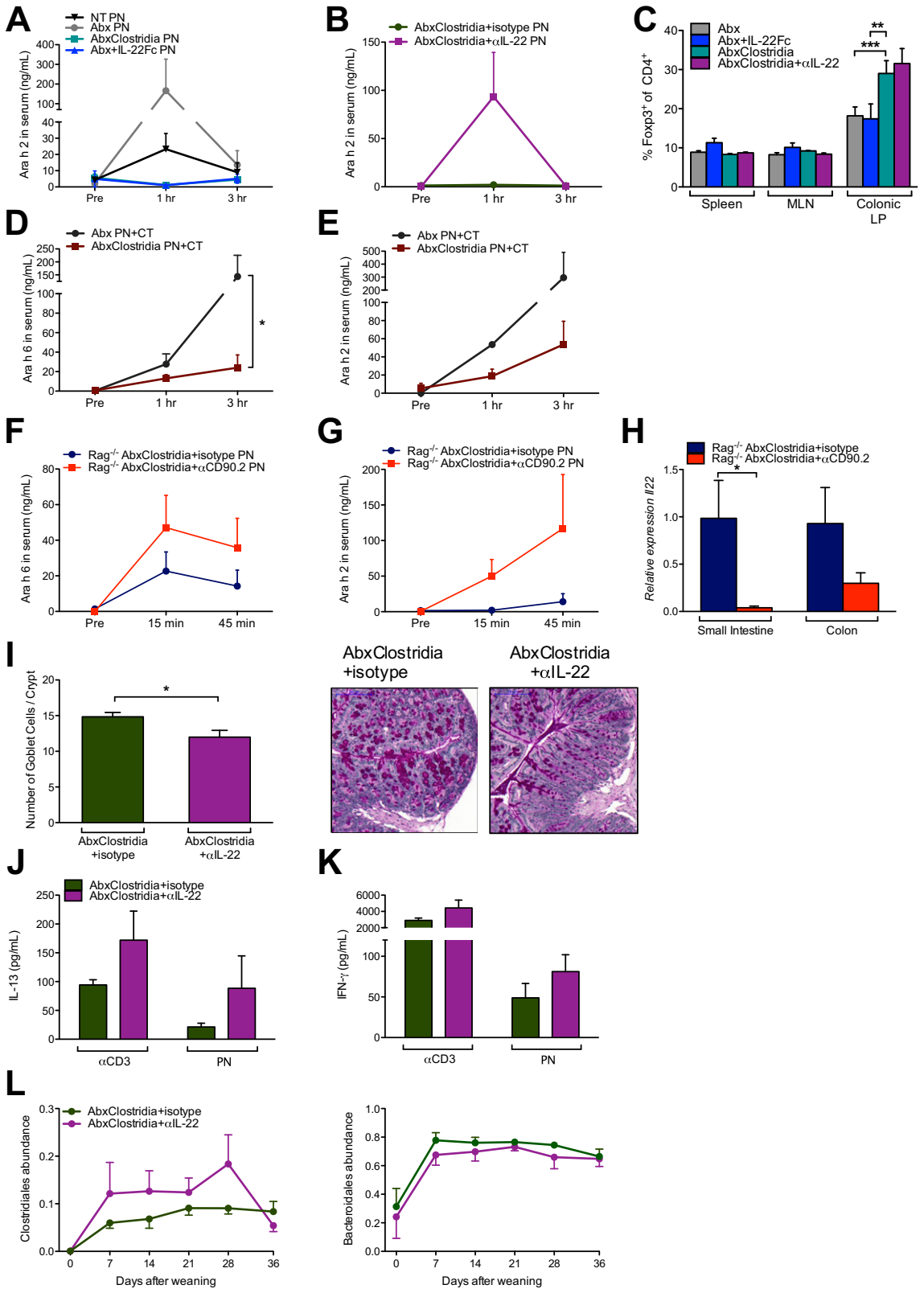

Fig. S4. (*A*) Serum Ara h 2 levels after PN gavage in NT or Abx-treated mice and Abx-treated mice colonized with Clostridia or given one i.p. injection of IL-22-Fc (mice from Fig. 5*B*; $n = 6$–9 mice per group) and (*B*) Abx-treated Clostridia colonized mice treated with neutralizing antibody to IL-22 or an isotype control (mice from Fig. 5*C*; $n = 5$–8 mice per group). (*C*) Flow cytometric analysis of Foxp3$^+$ Tregs among CD4$^+$ T cells in Abx mice with or without subsequent IL-22-Fc treatment, Clostridia colonization, or Clostridia colonization plus neutralizing antibody to IL-22 ($n = 3$–7 mice per group). (*D*) Serum levels of Ara h 6 and (*E*) Ara h 2 in Abx-treated mice or Abx-treated mice colonized with Clostridia and challenged with PN plus CT. $n = 4$ mice per group. (*F*) Serum Ara h 6 and (*G*) Ara h 2 after PN gavage in *Rag*$^{-/-}$ Abx-treated mice colonized with Clostridia and injected i.p. with anti-CD90.2 (ILC depleted) or isotype control. $n = 8$ mice per

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group. (*H*) Quantitative real-time PCR analysis of IL-22 transcripts from lamina propria lymphocytes isolated after colonization from Abx-treated Clostridia-colonized $Rag^{-/-}$ mice injected with anti-CD90.2 (ILC depleted) or isotype control (from *F* and *G*). Data are plotted relative to AbxClostridia+isotype and normalized to *Hprt*. $n = 8$ mice per group. (*I*) Quantification and representative images of goblet cells in distal colon of sensitized Abx-treated Clostridia-colonized mice treated with anti-IL-22 or an isotype control at 24 h after challenge (mice from Fig. 5 *D–I*). $n = 5$ mice per group. (Scale bar, 100 μm.) (*J*) Concentration of IL-13 and (*K*) IFN-γ in culture supernatants from splenocytes of mice in Fig. 5 *D–I*. (*L*) Abundance of Clostridiales and Bacteroidales for mice from Fig. 5 *D–I*. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ by two-way ANOVA with Bonferroni posttest (*C*, *D*, and *H*) or Student *t* test (*I*).