Supplementary Materials for

Prostaglandin E_2 constrains systemic inflammation through an innate lymphoid cell–IL-22 axis

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Materials and Methods

Mice.

Wild-type C57BL/6 mice were purchased from Harlan UK. To generate mice with selective EP4 deficiency in CD90.2^{+} ILCs, CD90.2(Thy1.2)-Cre mice on C57BL/6 background *(25)* were crossed to lox-flanked *Ptger4* (EP4-floxed) mice on C57BL/6 background *(26)*. EP4-floxed mice were also crossed with Lck-Cre mice (on C57BL/6 background) to generate mice with selective EP4 deletion in T cells *(27)*. All gene-modified mice including C57BL/6 *Rag1*$^{-/-}$ and IL-22$^{-/-}$ mice were bred and maintained under specific pathogen-free conditions in accredited animal facilities at the University of Edinburgh. Mice were aged >8 weeks old at the beginning of use and sex-matched. Both male and female mice were used in experiments. Mice were analyzed individually and no mice were excluded from the analysis, with the exception of exclusions due to technical errors in preparation of intestinal LPLs and failure of induction of Cre recombination by tamoxifen. All experiments were conducted in accordance with the UK Scientific Procedures Act of 1986 and had local ethical approval.

Treatment with small compounds, antibiotics, antibodies and rIL-22.

When indicated, mice were treated with indomethacin (5 mg/kg body weight/d, Sigma) or vehicle control (0.5% EtOH) in drinking water for 4-6 consecutive days for WT C57BL/6, *Rag1*$^{-/-}$ and IL-22$^{-/-}$ mice. In some experiments, mice were co-treated by intraperitoneal (i.p.) injection with an EP2 selective agonist (butaprost, Cayman) or an EP4 selective agonist (L-902,688, Cayman) at the dose of 10 μg per injection (one injection daily for 5 consecutive days) together with indomethacin from the starting day.

0.5% EtOH in PBS was used as vehicle control. As indicated, mice were treated with an EP4 selective antagonist (L-161,982 from Abcam, 10 mg/kg body weight/d) or vehicle control (0.5% DMSO) in drinking water for 6 consecutive days or through i.p. injection (20 μg per injection, one injection daily; vehicle control, 2% DMSO in PBS) for 5 consecutive days. To deplete ILCs, an anti-CD90.2 antibody (clone 30H12, BioXcell) or a rat IgG2b isotype control antibody (BioXcell) was injected i.p. into *Rag1*$^{-/-}$ mice at the dose of 250 μg per mouse every 3 days. Depletion efficiency was confirmed by flow cytometry. Recombinant IL-22 (PeproTech) was intravenously (i.v.) injected into *Rag1*$^{-/-}$ mice at the dose of 500 ng per mouse (one injection daily for 4 consecutive days) from the beginning of experiments. Control mice were injected i.v. with PBS. For antibiotic therapy, a cocktail of antibiotics containing ampicillin (0.5 mg/ml), gentamycin (0.5 mg/ml), metronidazole (0.5 mg/ml), neomycin (0.5 mg/ml), vancomycin (0.5 mg/ml) plus sucralose (4 mg/ml) was used as referenced to a previous report *(19)*. All antibiotics were purchased from Sigma and were administrated in drinking water together with indomethacin or vehicle for 5 d. When indicated, WT C57BL/6 mice were injected i.p. with lipopolysaccharides (LPS, Escherichia coli 0127:B8, Sigma) at a dose of 10 μg per mouse on d 5. Blood was withdrawn at 2 h after LPS injection or at the time that mice were culled. Serum was isolated following whole blood centrifugation and stored at -80^{o}C for analysis. In some experiments, mice were sacrificed 24 h after LPS/PBS injection and the peritoneal cavity lavaged (3 x 2 ml cold PBS) for neutrophil counts and lavage supernatant cytokines measurements. Other tissues such as spleen, liver, small and large intestines were then removed for further analysis.

Tamoxifen-induced EP4 deletion.

Induction of Cre recombination in CD90.2^{Cre}EP4$^{fl/+}$ mice by tamoxifen was described in a previous report *(25)*. Briefly, Tamoxifen (Sigma) was dissolved in sunflower oil/ethanol (10:1 ratio) at 10 mg/ml and injected i.p. into CD90.2^{Cre}EP4$^{fl/+}$ and control mice at the dose of 0.18 mg/g body weight/day for 3-5 consecutive days for Fig. 4B. Mice were injected i.p. with LPS (10 ug per injection per mouse) at 5 days after the last tamoxifen injection and blood was withdraw at 1.5 h after LPS injection. Splenic CD90.2^{+} cells were isolated using EasySep^{TM} Mouse CD90.2 Positive Selection Kit II (Stemcell technologies) and *Ptger4* gene expression was measured by real-time PCR in isolated splenic CD90.2^{+} cells to confirm the efficiency of conditional EP4 deletion. In Fig. S9C, tamoxifen was dissolved in sunflower oil excluding ethanol. Mice received tamoxifen by gavage at a dose of 0.18 mg/g body weight/day for five doses over seven days and were rested for further 4 days after the last tamoxifen treatment before being subjected to analysis.

DSS colitis.

WT C57BL/6 mice (8-9 weeks old at the beginning of experiments) were administrated with 2% (w/v) of dextran sulfate sodium (DSS, MW 36-50kDa, MP Biochemical) together with indomethacin (5 mg/kg body weight/d) or vehicle control (0.5% EtOH) in drinking water for 6 consecutive days. Recombinant IL-22 or PBS was injected i.p. into indicated mice at the dose of 500-1,000 ng per injection (one injection daily for 6 consecutive days) from the beginning of the experiments. Throughout the experimental timeline, mice were weighed and scored daily for a disease activity index (DAI) score to

allow for monitoring colitis progression and pathology. The DAI was scored by the following system *(37)*. Body weight: 0 (no or <1% weight loss compared to d 0 body weight), 1 (1-5% weight loss), 2 (5-10% weight loss), 3 (10-20% weight loss), and 4 (>20% weight loss); bleeding: 0 (no bleeding), 1 (blood present in/on faeces), 2 (visible blood in rectum), and 4 (visible blood on fur); and stool consistency: 0 (well formed/normal stool), 1 (pasty/semi-formed stool), 2 (pasty stool with some blood), 3 (diarrhea that does not adhere to anus), and 4 (diarrhea that does adhere to anus). Mice were immediately culled when body weight loss was greater that 25% or the total colitis score was 9 or higher.

Liver histology.

Liver samples were fixed with 10% neutral buffered formalin solution (Sigma), embedded in paraffin, and 5 μm sections were used for staining with hematoxylin and eosin (H&E). Adjustment of white balance was applied to the entire images.

Bacteria culture.

Counts of gut bacterial translocation to the liver were performed as described in a previous report *(19)*. Briefly, livers were removed under aseptic conditions and mechanically homogenized in sterile PBS. Tissue homogenates were serially diluted and cultured on LB agar (Sigma) at 37^{o}C for 2 days for subsequent colony counts.

Isolation of intestinal lamina propria cells.

Intestinal lamina propria (LP) cells were isolated as described previously *(38)*. In brief, mice were culled and their intestines removed and placed in cold PBS. After removing any remaining fatty and mesenteric tissues, samples were cut open longitudinally and any contents removed before being washed with HBSS buffer containing 2% FCS, and then cut into 0.5 cm pieces. Intestines were shaken at 37^{o}C for 15 min in HBSS containing 2 mM EDTA. After washing with pre-warmed HBSS buffer, intestines were again shaken at 37^{o}C for 30 min in HBSS containing 2 mM EDTA followed by wash with HBSS. Intestines were then transferred into gentleMACS C tubes (Miltenyi) digested in RPMI 1640 medium containing 10% FCS, 1% L-glutamine and antibiotics with 1.25 mg/ml collagenase IV (Roche) and 30 ug/ml DNase-I (Roche) by shaking at 37^{o}C for 30 min. Digested tissues were homogenized by gentleMACS disassociator running the programme m_intestine_01 and mashed through 40 μm cell strainer and flushed through HBSS containing 2% FCS. After centrifugation, cells were resuspended in complete RPMI 1640 medium for counting, staining, culture and/or sorting.

***In vitro* cell culture.**

MACS sorted small intestinal LP CD45^{+} cells, FACS sorted intestinal LP CD45^{+}Lin^{-} CD90.2^{+}KLRG1^{-}CCR6^{+} ILC3s, total *Rag1*$^{-/-}$ spleen cells, or FACS sorted splenic/bone marrow CD3^{-}CD11b^{-}CD11c^{-}CD4^{+} ILCs were cultured in completed RPMI 1640 medium (Gibco) supplemented with 10% FBS, 2-Mercaptoethanol (50 μM, Gibco), L-glutamine (2 mM, Gibco) and antibiotics (Penicillin and Streptomycin, 100 U/ml, Gibco). When indicated, recombinant IL-23 (20 ng/ml, eBioscience), IL-1β (20 ng/ml), IL-2 (10 ng/ml), IL-7 (10 ng/ml), indomethacin (10 μM, Sigma), PGE_2 (10 μM for detection of pCREB

and 100 nM for all other experiments except dose-response experiments, Cayman), an EP2 agonist butaprost (500 nM, Cayman), an EP4 agonist CAY10598 (500 nM, Cayman), an EP2 antagonist PF-04418948 (10 μM, Cayman), an EP4 antagonist L-161,982 (10 μM, Cayman), db-cAMP (300 μM, Sigma), 3-isobutyl-1-methylxanthine (IBMX, 500 μM for spleen cells and 100 μM for gut LPLs, Cayman), H-89 (10 μM, Merck), STAT3 inhibitor VI (S31-201, 25 μM, Merck), RORγt inhibitor (SR2211, 10 μM, Cayman), Ahr inhibitor (CH-223191, 10 μM, Merck) and their respective vehicle control (i.e. EtOH or DMSO) or combination of these reagents were added into cell cultures. For detecting cytokines in supernatants, cells were cultured for overnight or for 3 d as indicated in figure legends, and for intracellular staining of IL-22, cells were stimulated with IL-23 for 3-4 h in the presence of 0.2% GolgiPlug (BD Bioscience). *In vitro* cell cultures were performed in triplicates and repeated two or more times.

FACS sorting.

For purifying ILC3s from the small intestine of *Rag1*$^{-/-}$ mice, CD45^{+} LP cells were first isolated by MACS using mouse CD45 Microbeads (Miltenyi) and then stained with anti-CD45 eFluor450 or APC-Cy7 (clone 30-F11, eBioscience), anti-CD11c PE (clone N418, BioLegend), anti-CD11b PE (clone M1/70, eBioscience), anti-NK1.1 PE (clone PK136, eBioscience), anti-CD90.2 FITC or PE-Cy7 (clone 53-2.1, eBioscience), anti-KLRG1 APC (clone 2F1, eBioscience), anti-CCR6 Brilliant Violet (clone 29-2L17, Biolegend) on ice for 30 min. Alive CD45^{+}Lin^{-}(CD11c^{-}CD11b^{-}NK1.1^{-})CD90.2^{+}KLRG1^{-}CCR6^{+} ILC3s were sorted by FACSAria II (BD Bioscience). For purifying ILC3s from bone marrow and spleens of WT C57BL/6 mice, the CD4^{+} cell population was first isolated by MACS

with mouse CD4 MicroBeads (Miltenyi) and then stained with anti-CD3e PE (clone 145-2C11, eBioscience), anti-CD11b FITC (clone M1/70, BioLegend) and anti-CD4 PerCP-Cyanine5.5 (clone RM4-5, eBioscience) on ice for 30 min. In some experiments, an anti-CD11c PE (clone N418, BioLegend) was also used. Alive Lin^-($CD3^-CD11b^-CD11c^-$)$CD4^+$ cells were sorted by FACSAria II (BD Bioscience).

Surface and intracellular staining.

For surface staining, cells were first stained with the Fixable Viability Dye eFluor® 780 (eBioscience) on ice for 30 min to exclude dead cells. After wash, cells were stained on ice for another 30 min with indicated Abs such as anti-CD45 eFlour® 450, anti-CD3e PE, anti-CD11c PE, anti-CD11b PE, anti-B220 PE (clone RA3-6B2, eBioscience), anti-CD90.2 FITC, anti-CD4 PerCP-Cyanine5.5 or APC. Staining with Annexin V was performed using the FITC-Annexin V staining kit (Biolegend). For intracellular staining of IL-22, cells were stimulated with IL-23 (20 ng/ml) for 3-4 h in the presence of GolgiPlug (BD Bioscience). After staining with the Fixable Viability Dye eFluor® 780 and surface markers, cells were fixed by the BD Cytofix/Cytoperm Fixation buffer (BD Bioscience) for 30 min and then stained with anti-human/mouse IL-22 APC (clone IL22JOP, eBioscience) in the BD Perm/Wash Buffer on ice for 1 h. For staining of RORγt, a Foxp3/Transcription Factor Fix and Staining Buffer (eBioscience) and Anti-Mouse ROR-γt PerCP-eFluor710 (clone B2D, eBioscience) were used. For staining of peritoneal $CD11b^+$Ly-$6G^+$ neutrophils, peritoneal cells were first stained with Fixable Viability Dye eFluor® 780 on ice for 30 min. After wash, cells were stained on ice for another 30 min with anti-CD45 eFlour® 450, anti-CD11b FITC and anti-Ly-6G (Gr-1)

APC (clone RB6-8C5, eBioscience). To stain p-CREB, cells were stimulated with 10 uM of PGE_2 or vehicle at 37°C for 30 min, then fixed by a Foxp3/Transcription Factor Fix buffer (eBioscience) for overnight followed by staining with surface markers and a Phospho-CREB (Ser133) XP Rabbit mAb (clone D1G6, Cell Signaling) or a normal rabbit IgG for 1 h. After washing, cells were incubated with AF680 Donkey anti-Rabbit IgG for another 30 min. To stain pSTAT3, fresh spleen cells from *Rag1*$^{-/-}$ mice were cultured with PGE_2 or vehicle for 5 h and then stimulated with IL-23 for 15 min. Cells were fixed by the Foxp3/Transcription Factor Fix buffer for overnight followed by staining with surface markers and Alexa Fluor 488 Mouse Anti-STAT3(pS727) (clone 49, BD Bioscience). Flow cytometry was performed on the BD LSRFortessa (BD Bioscience) and analyzed by FlowJo software (Tree Star).

Enzyme-linked immunosorbent assay (ELISA).

For detection of cytokines in serum and supernatants of cell cultures or peritoneal lavage fluids, commercial ELISA kits were used according to the manufacturers' instructions. ELISA Ready-SET-Go!® kits for mouse IL-22 and IL-6 were obtained from eBioscience, while the Mouse TNF-α ELISA MAX™ Deluxe was purchased from BioLegend.

Gene expression and Real-time PCR.

RNA purification from sorted ILC3s or homogenized terminal ileum tissues was performed by using the Rneasy Mini Kit (Qiagen). cDNA was obtained by reverse transcription using the High-capacity cDNA Reverse Transcription Kits (ABI). Samples were analyzed by real-time PCR with SYBR Premix Ex Taq II (Tli RNase H Plus) kit

(Takara) or GoTaq qPCR Master Mix (Promega) on the Applied Biosystem 7900HT Fast machine. Below primers (some were obtained from ref. *39-47*) were used**.** *Il22* forward, 5'-CATGCAGGAGGTGGTACCTT-3'; *Il22* reverse, 5'-CAGACGCAAGCATTT CTCAG-3'. *Il22ra1* forward, 5'-TGCTCTGTTATCTGGG CTACAA-3'; *Il22ra1* reverse, 5'-TCAGGACACGTTGGACGTT-3'. *Il10rb* forward, 5'-TCTCTTCCACAGCACCT GAA-3'; *Il10rb* reverse, 5'-GAACACCTCGGCCTCCTC-3'. *Reg3b* forward, 5'-TGGGAATGGAGTAAAATG-3'; *Reg3b* reverse, 5'-GGCAACTTCACCTCACAT-3'. *Reg3g* forward, 5'-CCATCTTCACGTAGCAGC-3'; *Reg3g* reverse, 5'-CAAGATGTCCT GAGGGC-3'. *Muc1* forward, 5'-GGTTGCTTTGGCTATCGTCTATTT-3'; *Muc1* reverse, 5'-AAAGATGTCCAGCTGCCCATA-3'. *Muc3* forward, 5'-CGTGGTCAACT GCGAGAATGG-3'; *Muc3* reverse, 5'-CGGCTCTATCTCTACGCTCTCC-3'. *Fut2* forward, 5'-TGTGACTTCCACCATCATCC-3'; *Fut2* reverse, 5'-TCTGACAGGGTTT GGAGCTT-3'. *Cldn1* forward, 5'-GAGGGACTGTGGATGTCCTG-3'; *Cldn1* reverse, 5'-ATGCCAATTACCATCAAGGC-3'. *Cldn2* forward, 5'-TGAACACGGACCACTGA AAG-3'; *Cldn2* reverse, 5'-TTAGCAGGAA GCTGGGTCAG-3'. *Ocln* forward, 5'-CATAGTCAGATGGGGGTGGA-3'; *Ocln* reverse, 5'-ATTTATGATGAACAGCCCCC-3'. *F11r* forward, 5'-CTGATCTTTGACCCCGTGAC-3'; *F11r* reverse, 5'-ACCAGACG CCAAAAATCAAG-3'. *Tjp1* forward, 5'-ACTCCCACTTC CCCAAAAAC-3'; *Tjp1* reverse, 5'-CCACAGCTGAAGGACTCACA-3'. *Tjp2* forward, 5'-CGGATTCCAGAC AAGGTGTT-3'; *Tjp2* reverse, 5'-ATTCACGTTGATTGTGGCTG-3'. *Tnfsf11* forward, 5'-CGCTTCCCGATGTTTCATG-3'; *Tnfsf11* reverse, 5'-GGTTAACCAAGATGG CTTCTATTACC-3'. *Tnfsf4* forward, 5'-GGGAAGGGGTTCAACCCCTGG-3'; *Tnfsf4* reverse, 5'-GAAGAGAGTTGCAGGCAGACA-3'. *Il17a* forward, 5'-TGTGAAGGTC

AACCTCAAAGTC-3'; *Il17a* reverse, 5'-GAGGGATATCTATCAGGGTCTTCA-3'. *Il23r* forward, 5'-CCAAGTATATTGTGCATGTGAAGA-3', *Il23r* reverse, 5'-AGCTT GAGGCAAGATATTGTTGT-3'. *Il1r1* forward, 5'-TGGAACAGAGCCAGTGTCAG-3'; *Il1r1* reverse, 5'-CAGGAGAAGTCGCAGGAAGT-3'. *Lta* forward, 5'-GTACCCAA CAAGGTGAGCAGC-3'; *Lta* reverse, 5'-CCAGGACAGCCCATCCACT-3'. Ltb forward, 5'-ACGCTTCTTCTTGGCTCGC-3'; Ltb reverse, 5'-ACCTCATAGGCGCTT GGATG-3'. *Il7ra* forward, 5'-GGAACAACTATGTAAGAAGCCAAAAACG-3'; *Il7ra* reverse, 5'-AAGATCATTGGGCAGAAAACTTTCC-3'. *Ccr6* forward, 5'-CCTCACA TTCTTAGGACTGGAGC-3'; *Ccr6* reverse, 5'-GGCAATCAGAGCTCTCGGA-3'. *Csf2* forward, 5'-GCATGTAGAGGCCATCAAAGA-3'; *Csf2* reverse, 5'-CGGGTCTGCACA CATGTTA-3'. *Bcl2* forward, 5'-AGTACCTGAACCGGCATCTG-3'; *Bcl2* reverse, 5'-AGGTATGCACCCAGAGTGATG-3'. *Bcl2l1* forward, 5'-ATGACCACCTAGAGCC TTGGA-3'; *Bcl2l1* reverse, 5'-GAAGAGTGAGCCCAGCAGAAC-3'. *Ptger4* forward, 5'-CCTAACCCCACCCTACAGGT-3'; *Ptger4* reverse, AGAAGGACGCGTTGAC TCC-3'. *Gapdh* forward, 5'-TGAACGGGAAGCTCACTGG-3'; *Gapdh* reverse, 5'-TCCACCACCCTGTTGCTGTA-3'. Expression of genes *Rorc* (Mm01261022_m1), *Ahr* (Mm00478932_m1), *Stat3* (Mm01219775_m1), *Id2* (Mm00711781_m1), *Tcf7* (Mm00493445_m1), *Zbtb16* (Mm01176868_m1), *Tox* (Mm00455231_m1), *Nfil3* (Mm00600292_s1) were measured by Taqman Gene Expression Master Mix (Life Technologies) with respective primer sets obtained from Life Technologies. Expression was normalized to mouse glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*) and presented as relative expression to control group by the $2^{-\Delta\Delta Ct}$ method.

Gene expression analysis of neonatal sepsis blood cells.

Gene transcription levels were measured by transcription microarray in a study by Smith *et al. (15)* on human neonates comparing confirmed bacterial infection (n=27 independent neonates) with non-infection controls (n=35 independent neonates). Processed and normalized data for genes *PTGES2* and *PTGER4* were retrieved from this study and probability density functions computed for each gene, per group. For each gene, the hypothesis of differential expression between infected and control neonates was tested through a Wilcoxon-Rank-Sum test. A separate hypothesis of different inter-subject variability was tested for through Fligner-Killeen tests. Data of blood neutrophil counts in neonatal sepsis and control patients were retrieved from our previous report *(15)* to complete a new analysis work for investigating their association with *PTGES2* and *PTGER4* gene expression by non-parametric Spearman correlation test.

Human ILC3 sort and culture and human IL-22 analysis.

Human $Lin^{-}CD161^{+}CD127^{+}CRTH2^{-}CD117^{+}$ ILC3s were sorted from peripheral blood of healthy donors. Lineage markers used for sorting included CD1a (clone HI149), CD3 (clone OKT3), CD14 (clone HCD14), CD34 (clone 581), CD94 (clone DX22), CD123 (clone 6H6), TCRα/β (clone IP26), TCRγ/δ (clone B1), BDCA2 (201A) and FcεR1 (clone AER-37). Anti-human CD161 (clone HP-3G10), CD127 (clone A019D5), CD117 (clone 104D2) and CD294/CRTH2 (clone BM16) were also used. All these Abs were obtained from Biolegend except CD294, which was purchased from BD Bioscience. Sorted human ILC3s were cultured with recombinant human IL-2 (5 ng/ml, Biolegend), human IL-23 (50 ng/ml, R&D system) and human IL-1β (50 ng/ml, Biolegend) for 4

days to induce IL-22 production. PGE_2 (100 nM) or vehicle was added into the culture system from the starting of culture. IL-22 levels in supernatants was measured by Human IL-22 ELISA MAX Deluxe (Biolegend).

Acute pancreatitis patients.

Human plasma samples were obtained with ethical approval and fully-informed consent (MREC ref. number 13/SS/0136, United Kingdom). Samples from our clinical archive obtained from persons presenting to hospital with acute pancreatitis (AP) over a three-month continuous period in 2013 (UKCRN ID: 16116) (http://public.ukcrn.org.uk/Search/StudyDetail.aspx?StudyID=16116) were included. The diagnosis of AP was made according to the Revised Atlanta Criteria 2012 *(48)*, namely an elevated serum amylase concentration > 300 IU/L in the context of abdominal pain, nausea and/or vomiting. Healthy volunteer blood samples were collected with ethical approval and fully-informed consent (REC ref. number 08/S1103/38, United Kingdom). Plasma aliquots were stored at -80^{o}C for <2 years with a single thaw before use. IL-22 concentrations were measured by ELISA as described above.

Statistical analysis.

All data were expressed as mean ± SEM. Statistical significance between two groups was examined by the Student's *t*-test or the Mann-Whitney test, while the one-way and two-way analysis of variance (ANOVA) with Bonferroni's, Holm-Sidak's or Newman-Keuls multiple comparisons test were used to evaluate multiple groups. Correlation analysis Fig. 3E and Fig. S13 was calculated by Pearson's correlation coefficient (r). Statistical

work was performed using Prism 6 software (GraphPad) and a *P* value of less than 0.05 was considered as significance.

Author Contributions

R.D., R.A.O., S.C., C.Yu, C.R., F.R. and C.Yao performed experiments. D.S., S.T., J.R., A.P., R.B.W., R.M.B., J.P.I., S.M.A., S.N. and R.M.M. provided technical expertise, essential reagents, transgenic mice and editorial advice. X.Z., C.S. and D.M. provided human plasma samples of AP patients and analyzed IL-22 levels. T.F. and P.G. performed work on neonatal sepsis and analysis of PG receptor expression on ILC3s. R.D., S.E.H., A.G.R. and C.Yao wrote the manuscript. S.E.H., A.G.R. and C.Yao conceived this project and supervised the research.

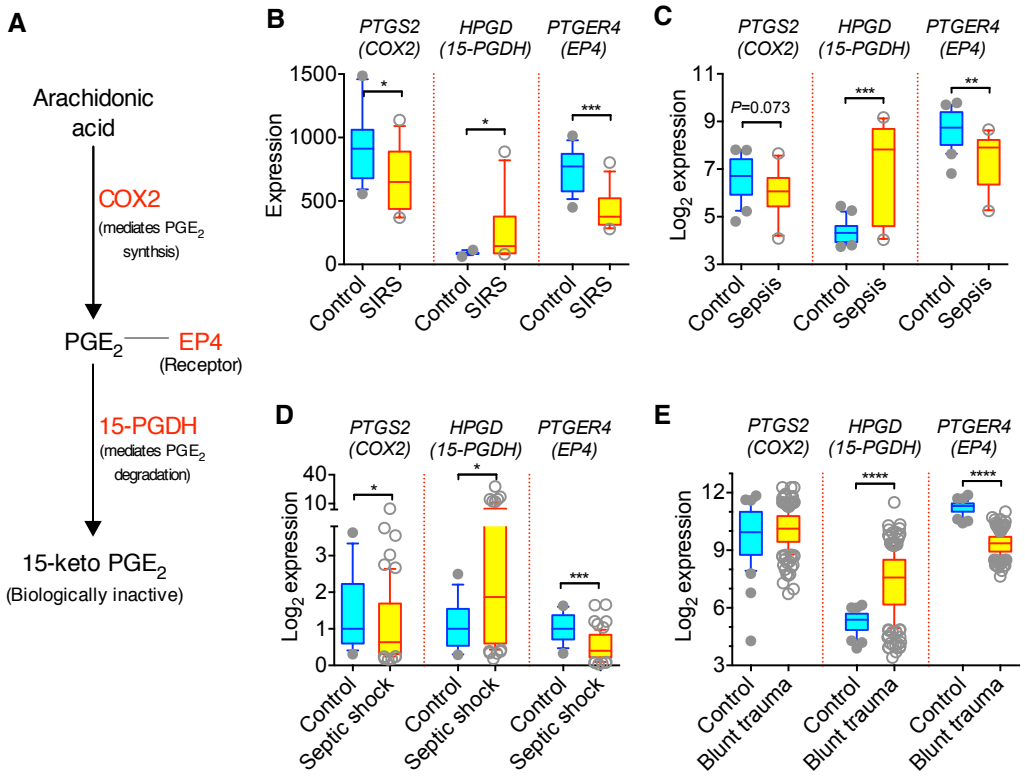

Fig. S1. Down-regulation of PGE_2-EP4 signaling is associated with development of severe systemic inflammatory diseases in humans. (A) A schematic chart indicates PGE_2 synthesis from arachidonic acid by COX2 (encoding by *PTGS2*) and degradation into biologically inactive 15-keto PGE_2 by 15-PGDH (encoding by *HPGD*). The PGE_2 receptor EP4 (encoding by *PTGER4*) is also shown. **(B)** Expression of *PTGS2*, *HPGD* and *PTGER4* in blood cells taken from patients diagnosed with systemic inflammatory response syndrome (SIRS, n=12) or healthy control individuals (n=18) on d 1. Data were retrieved from the Gene Expression Omnibus dataset GSE40012 *(49)*. **(C)** Expression of *PTGS2*, *HPGD* and *PTGER4* in blood cells taken from patients with sepsis (n=10) or healthy control (n=20). Data were retrieved from the Gene Expression Omnibus dataset GSE28750 *(50)*. **(D)** Expression of *PTGS2*, *HPGD* and *PTGER4* in blood cells taken from normal children (n=18) or children with septic shock (n=67) on d 1. Data were retrieved from the Gene Expression Omnibus dataset GSE13904 *(51)*. **(E)** Expression of *PTGS2*, *HPGD* and *PTGER4* in blood cells taken from healthy individuals (n=37) or patients with severe blunt trauma or burn injury (n=205) within 24 h from injury. Data were retrieved from the Gene Expression Omnibus dataset GSE36809 *(52)*. **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001 by 2-tailed Mann-Whitney test.

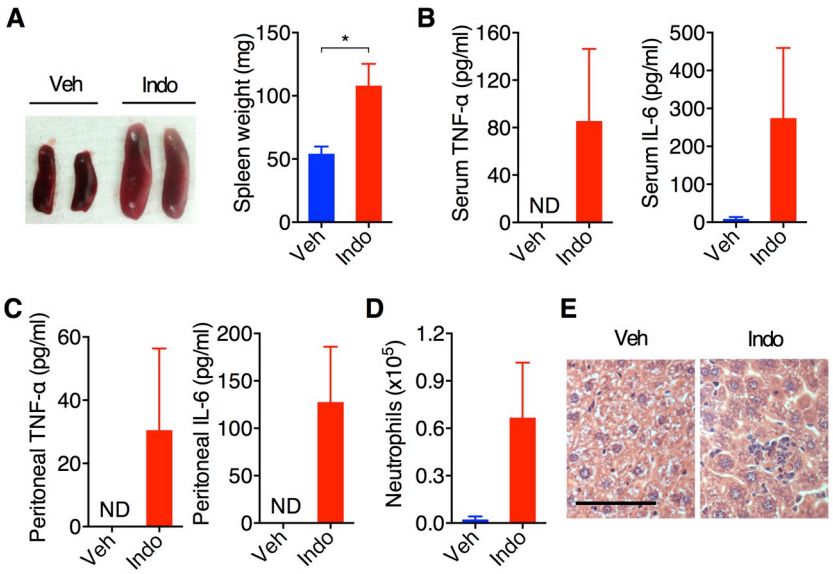

Fig. S2. Adaptive immune cells are not required for indomethacin-dependent systemic inflammation. *Rag1*$^{-/-}$ mice were treated with indomethacin (n=5) or vehicle control (n=4) in drinking water for 4 d. **(A)** Spleen size and weight. **(B)** IL-6 and TNF-α concentrations in serum. **(C)** IL-6 and TNF-α concentration in peritoneal cavity lavage fluids. **(D)** Accumulation of CD11b^{+}Ly-6G^{+} neutrophils in peritoneal cavities. **(E)** H&E staining histological sections of the liver. Data shown as mean ± SEM are pooled from two independent experiments. Scale bar, 50 μm. **P*<0.05 by the unpaired, 2-tailed student *t*-test. ND, not detected.

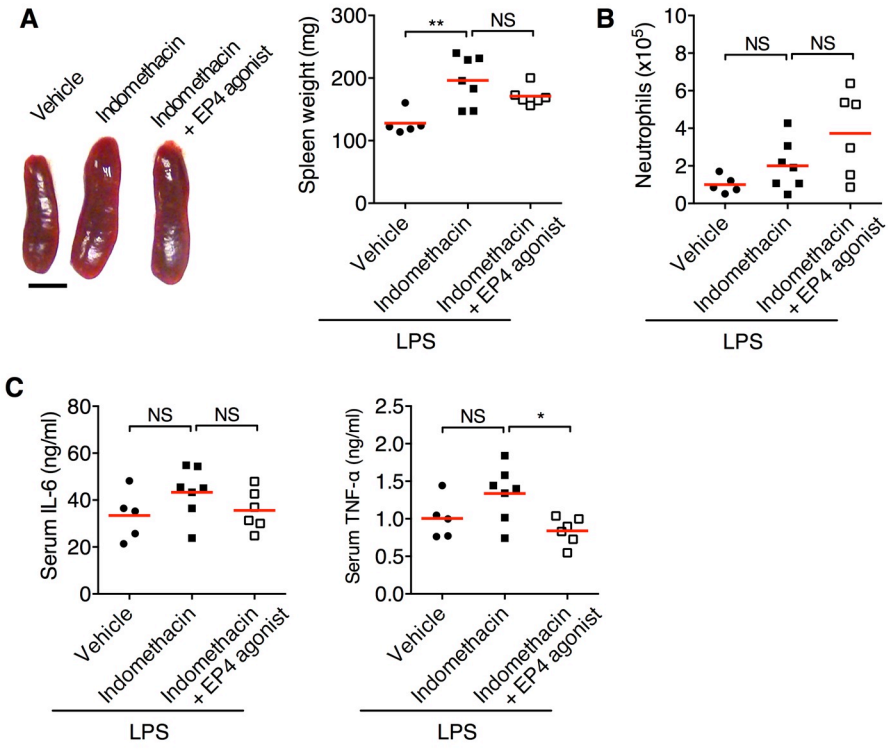

Fig. S3. IL-22 is required for control of systemic inflammation by PGE_2–EP4 signaling. Spleen size and weight (**A**), neutrophils in peritoneal cavity (**B**) and serum levels of TNF-α and IL-6 (**C**) of C57BL/6 IL-$22^{-/-}$ mice treated with indomethacin in drinking water plus administration (i.p.) of an EP4 agonist or vehicle control for 5 d followed by LPS challenge (i.p.) for another 24 h (A and B) or 2 h (C). Each point represents one mouse. Data are pooled from two independent experiments. Scale bar, 0.5 cm. **P*<0.05, ***P*<0.01 by one-way ANOVA. NS, not significant.

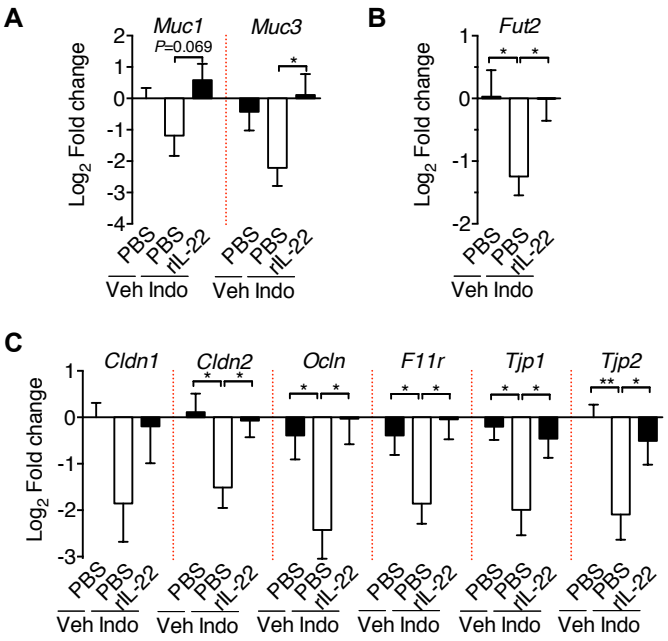

Fig. S4. Gene expression profile of intestinal barrier function. *Rag1*$^{-/-}$ mice treated with vehicle (n=9) or indomethacin in drinking water plus administration (i.v.) of rIL-22 (n=10) or PBS (n=10) for 5 d as in Fig. 3, C to G. **(A** to **C)** Gene expression of mucins (A), fucosylthransferase 2 (B) and tight junctions (C) in the terminal ileum. Expression was normalized to *Gapdh* gene and presented as relative expression (in Log_2 Fold Change) to the vehicle/PBS control group. Data shown as means ± SEM are pooled from three independent experiments. **P*<0.05, ***P*<0.01 by one-way ANOVA.

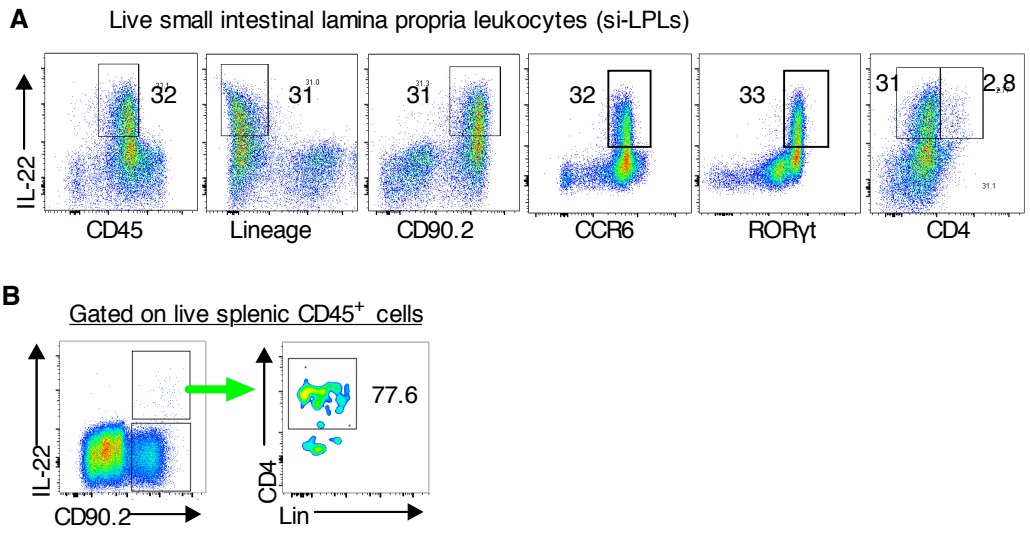

Fig. S5. Identification of IL-22-producing type 3 innate lymphoid cells in the gut and spleen. (A) Intracellular staining of small intestinal lamina propria lymphocytes (LPLs) from *Rag1*$^{-/-}$ mice cultured with IL-23 for 4 h. The result indicates that IL-22-expressing innate cells in the gut are CD45$^{+/Low}$Lineage^{-}(CD11c/CD11b/NK1.1)CD90.2^{+}CCR6^{+} RORγt^{+} ILC3s and most of these cells (>90%) do not express CD4. **(B)** Intracellular staining of total *Rag1*$^{-/-}$ spleen cells cultured with IL-23 for 4 h. The result indicates that all IL-22-expressing innate cells in the spleen are CD45^{+}Lin^{-}CD90.2^{+} and the majority (70-80%) of them are CD4^{+} ILC3s.

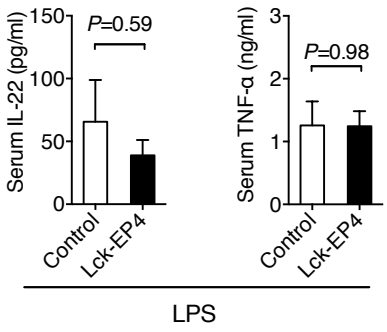

Fig. S6. PGE_2–EP4 signaling in T cells is not required for control of systemic inflammation. Serum levels of IL-22 and TNF-α of mice with specific EP4 deletion in T cells (i.e., Lck-EP4 mice mixed of $Lck^{Cre}EP4^{fl/+}$ and $Lck^{Cre}EP4^{fl/fl}$ mice, n=4) and control litter mates (mixed of Lck^{Cre}, $EP4^{fl/fl}$, $EP4^{fl/+}$ and $EP4^{+/+}$ mice, n=8) at 1.5 h after LPS challenge. Efficiency of EP4 deletion in T cells of $Lck^{Cre}EP4^{fl/+}$ (~50%) and $Lck^{Cre}EP4^{fl/fl}$ (~97%) mice comparing to control mice has been shown previously *(27)*. Data shown as means ± SEM are pooled from two independent experiments. *P* values are calculated by unpaired two-tailed Student's *t*-test.

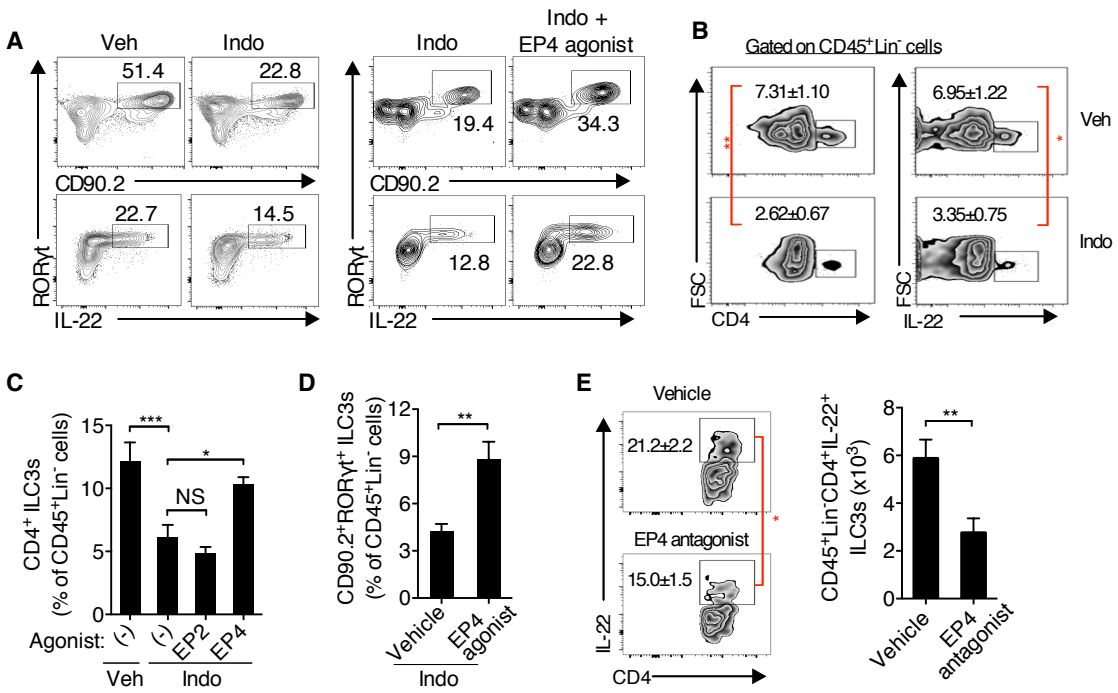

Fig. S7. PGE_2–EP4 signaling regulates innate lymphoid cell homeostasis in spleen. **(A)** Intracellular staining of small intestinal LPLs of C57BL/6 *Rag1*$^{-/-}$ mice treated with vehicle (n=9) or indomethacin in drinking water plus an EP4 agonist (n=8) or vehicle (n=10) for 4 days as in Fig.4C. Representative dot plot data show RORγt$^+$IL-22$^+$ cells within alive CD45$^+$ populations. **(B)** Frequencies of CD45$^+$Lin$^-$CD4$^+$ and CD45$^+$Lin$^-$IL-22$^+$ ILC3s in spleens of *Rag1*$^{-/-}$ mice treated with indomethacin (Indo, n=8) or vehicle (Veh, n=6) for 4 d. **(C)** Frequencies of CD45$^+$Lin$^-$CD4$^+$ ILC3s in spleen of *Rag1*$^{-/-}$ mice treated with vehicle (n=6) or indomethacin plus administration (i.p.) of agonist for EP2 (n=6), EP4 (n=6) or vehicle control (n=8) for 4 days. **(D)** Frequencies of CD45$^+$Lin$^-$ CD90.2$^+$RORγt$^+$ ILC3s in spleen of *Rag1*$^{-/-}$ mice treated with indomethacin plus administration (i.p.) of an EP4 agonist or vehicle control (n=8 per group) for 4 days. **(E)** Frequencies and numbers of CD45$^+$Lin$^-$CD4$^+$IL-22$^+$ ILC3s in spleens of *Rag1*$^{-/-}$ mice treated (i.p.) with an EP4 antagonist (n=10) or vehicle (n=9) for 4 d. Data shown as mean ± SEM are pooled from two independent experiments. **P*<0.05, ***P*<0.01, ****P*<0.001 by Mann-Whitney test (B, D and E) or one-way ANOVA (C).

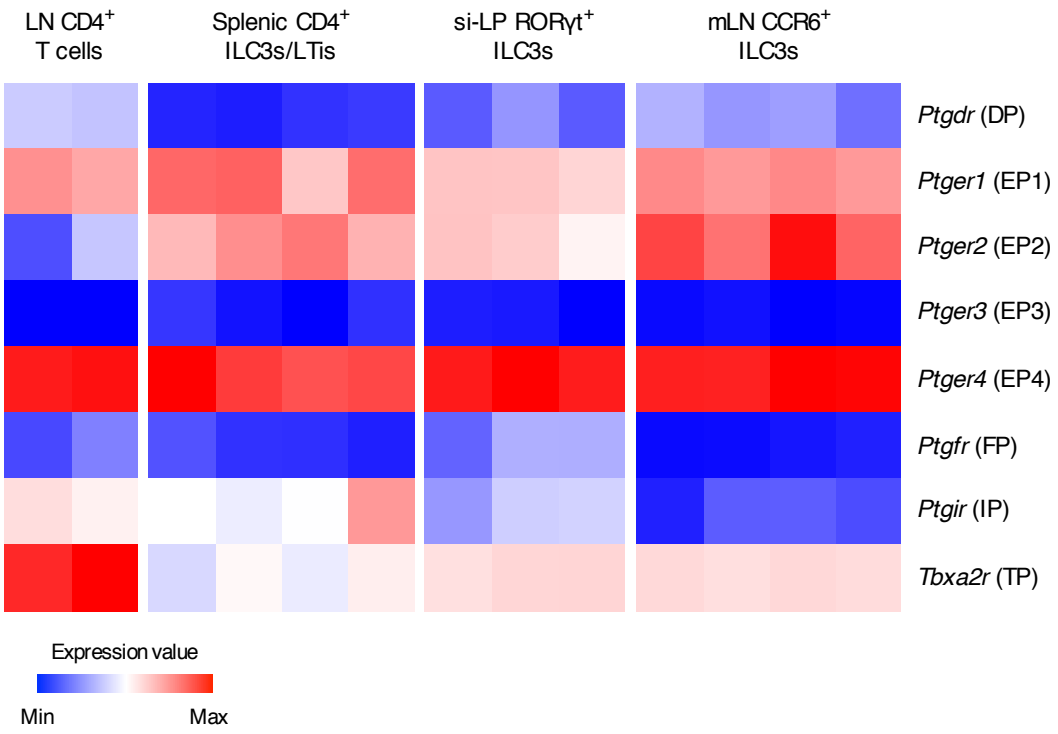

Fig. S8. Expression of PG receptors by ILC3s. Gene expression of PG receptors in FACS-sorted ILC3 populations isolated from various tissues including splenic Lin^- $CD90.2^+CD25^+CD4^+$ ILC3s/LTis (Lymphoid tissue inducer cells, n=4), small intestinal LP (si-LP) $CD45^+Lin^-NKp46^+ROR\gamma t^{hi}$ ILC3s (n=3), and mesenteric lymph node (mLN) $Lin^-CD90.2^+CD127^+CCR6^+ST2^-$ ILC3s (n=4). Gene expression in lymph node (LN) $TCR^+CD4^+CD8^-CD25^-CD62^{hi}CD44^{low}$ naïve T cells (n=2) was used as control. Results suggest that in ILC3s the PGE_2 receptor EP4 (encoded by *Ptger4*) is the most expressed among 8 subtypes of PG receptors. While ILC3s may also express PGE_2 receptors EP1 (encoded by *Ptger1*) and EP2 (encoded by *Ptger2*) and thromboxane A2 receptor TP (encoded by *Tbxa2r*), ILC3s are unlikely to express PGD_2 receptor DP (encoded by *Ptgdr*), PGE_2 receptor EP3 (encoded by *Ptger3*), $PGF_{2\alpha}$ receptor FP (encoded by *Ptgfr*) and PGI_2 receptor IP (encoded by *Ptgir*). Data were retrieved from the Gene Expression Omnibus dataset GSE37448 for LN $CD4^+$ T cells and si-LP $ROR\gamma t^+$ ILC3s *(53),* GSE46468 for splenic $CD4^+$ ILC3s/LTis *(54)*, and GSE67076 for mLN $CCR6^+$ ILC3s *(55)*.

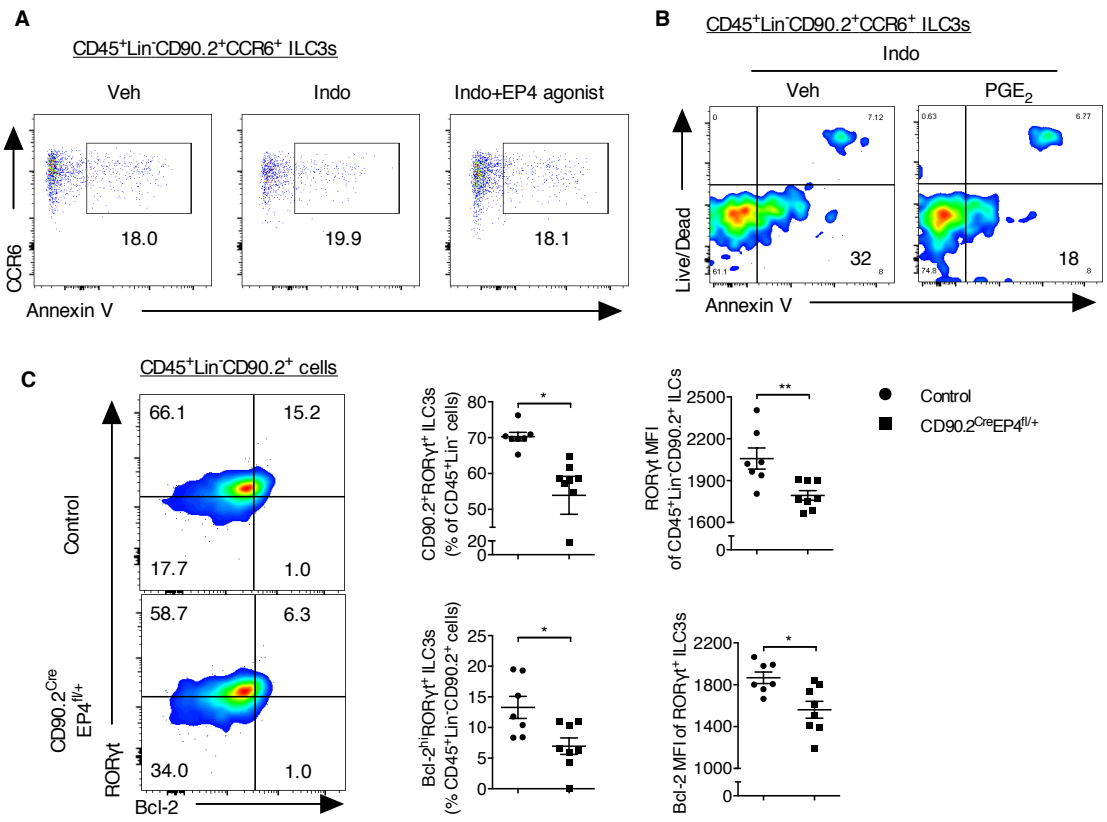

Fig. S9. PGE_2 prevents ILC3 apoptosis and augments IL-7 responsiveness *in vitro*. **(A)** Expression of Annexin V in $CD45^+Lin^-CD90.2^+CCR6^+$ ILC3s from mesenteric lymph nodes of C57BL/6 mice treated with vehicle (n=4) or indomethacin in drinking water plus an EP4 agonist (n=7) or vehicle (n=5) for 5 days. **(B)** Mesenteric lymph node cells of *Rag1*$^{-/-}$ mice were cultured *in vitro* with indomethacin in the presence or absence of PGE_2 for 24 h. Data showing expression of Annexin V in $CD45^+Lin^-CD90.2^+CCR6^+$ ILC3s are representative of two independent experiments. **(C)** Mesenteric lymph node cells from tamoxifen-treated $CD90.2^{Cre}EP4^{fl/+}$ (n=8) or control (n=7) mice were cultured *in vitro* with IL-7 for 24 h. Expression of Bcl-2 and RORγt in $CD45^+Lin^-CD90.2^+$ ILCs were shown. **P*<0.05, ***P*<0.01 by two-tailed unpaired Student's *t*-test.

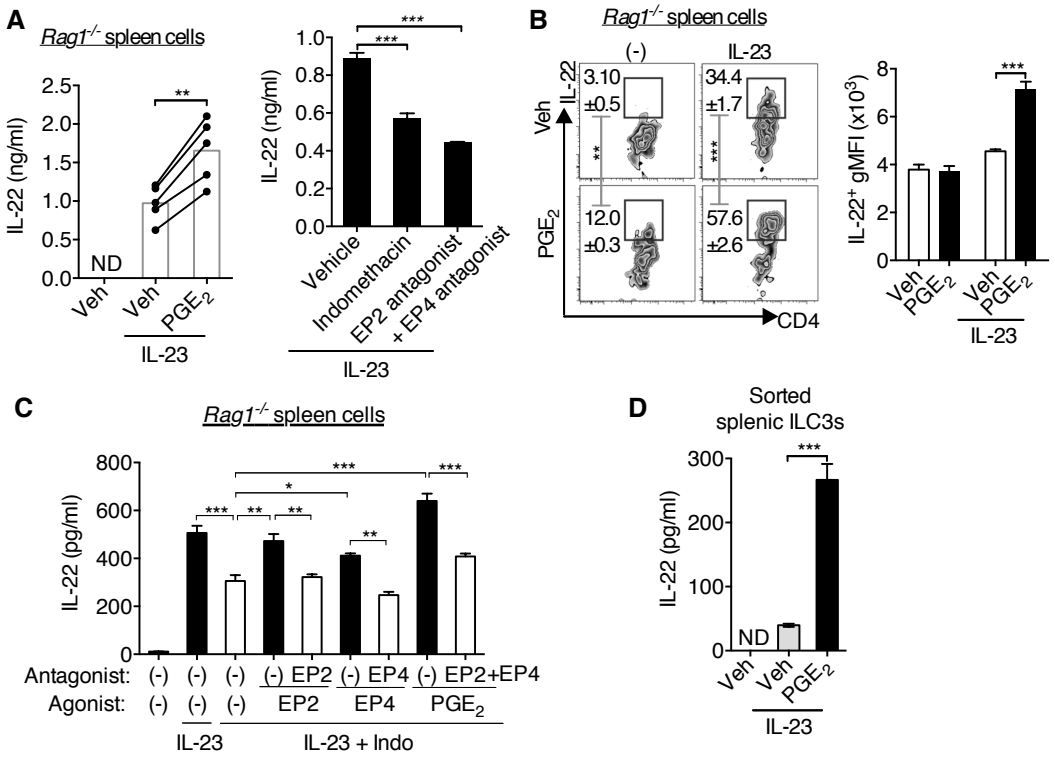

Fig. S10. PGE_2 promotes IL-22 production from splenic ILC3s via EP2 and EP4 receptors *in vitro*. (A) IL-22 levels in supernatants of total *Rag1*$^{-/-}$ spleen cells cultured with IL-23 and PGE_2, indomethacin or EP2 antagonist plus EP4 antagonist overnight. **(B)** Intracellular IL-22 expression in Lin$^-$(CD3$^-$B220$^-$Ter119$^-$CD11c$^-$)CD4$^+$ cells of *Rag1*$^{-/-}$ spleen cells cultured under indicated conditions for 4 h. **(C)** IL-22 levels in supernatants from total *Rag1*$^{-/-}$ spleen cells cultured with IL-23 without or with indomethacin plus agonists and/or antagonists for EP2 or EP4 as indicated overnight. **(D)** IL-22 production by Lin$^-$CD4$^+$ ILC3s sorted from spleen and/or bone marrow and then cultured with IL-23 in the presence or absence of PGE_2 for 3 d. Data shown as means ± SEM are representative of two (B-D) or three (A, right) or pooled from five (A, left) independent experiments. **P*<0.05, ***P*<0.01, ****P*<0.001 by one-way ANOVA (A right, B and C), unpaired (D) or paired (A left) Student's *t*-test or. ND, not detected.

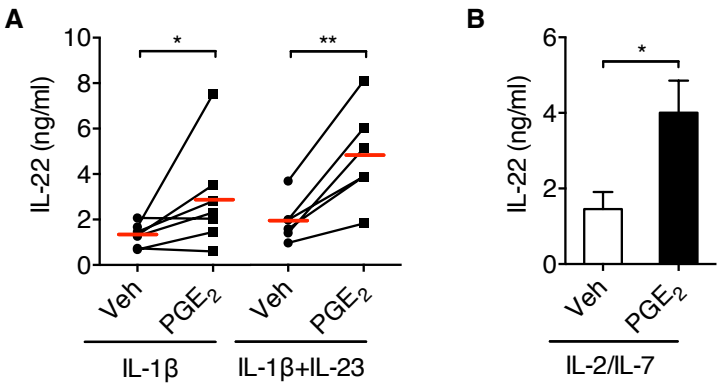

Fig. S11. Cytokine response of ILC3s regulated by PGE_2. IL-22 levels in supernatants of LPLs isolated from small intestines of *Rag1*$^{-/-}$ mice and then cultured with indicated cytokines in the presence or absence of PGE_2 overnight (A) or for 3 days (B). Data shown as means ± SEM are pooled from 2-3 independent experiments. For the panel A, n=6-7 biological replicates and for the panel B, n=4 biological replicates. **P*<0.05, ***P*<0.01 by 2-way ANOVA (A) or unpaired Student's *t*-test (B).

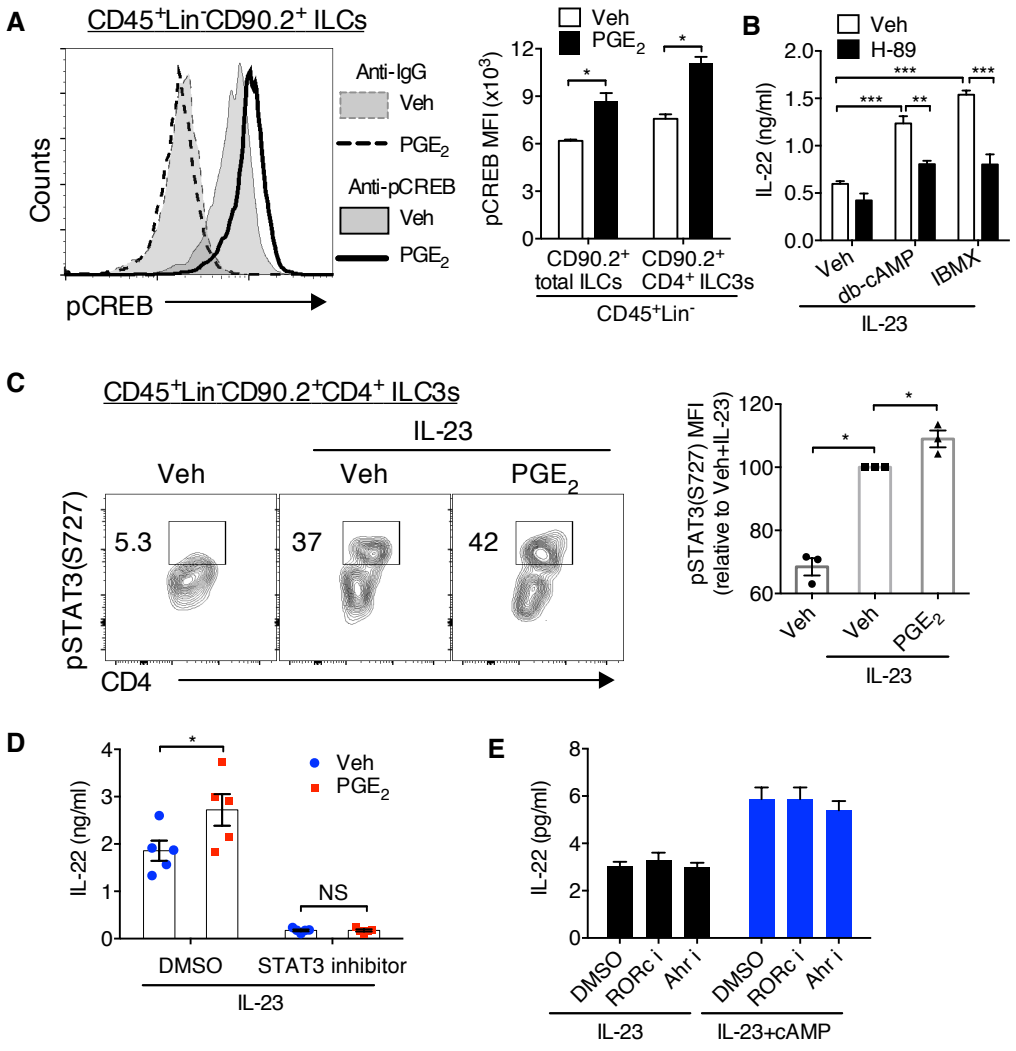

Fig. S12. Cyclic AMP-PKA and IL-23-STAT3 (transcription factor signal transducer and activator of transcription) signaling pathways mediate innate IL-22 production *in vitro*. **(A)** Intracellular expression of phosphorylated CREB (pCREB) in splenic $CD45^+Lin^-CD90.2^+$ ILCs and $CD45^+Lin^-CD90.2^+CD4^+$ ILC3s stimulated with PGE_2 or vehicle for 30 min. MFI, mean fluorescence intensity. Data shown as means ± SEM are representative of two independent experiments. **(B)** IL-22 production in supernatants from total *Rag1*$^{-/-}$ splenocytes cultured with IL-23 plus db-cAMP or IBMX with or without H-89 overnight. db-cAMP, dibutyryl-cAMP; IBMX, 3-isobutyl-1-methylxanthine. Data shown as means ± SEM are representative of two independent experiments. **(C)** Intracellular expression of phosphorylated STAT3 (pSTAT3) in splenic $CD45^+Lin^-CD90.2^+CD4^+$ ILC3s stimulated with PGE_2 or vehicle for 5 h and then restimulated with IL-23 for a further 15 min. Data shown as means ± SEM are pooled from three independent experiments. **(D)** IL-22 production in supernatants from small intestinal LPLs of *Rag1*$^{-/-}$ mice cultured with IL-23 with or without PGE_2 and a STAT3

inhibitor overnight. Data shown as means ± SEM (n=5 biological replicates). **(E)** IL-22 production in supernatants from total *Rag1*$^{-/-}$ splenocytes cultured with IL-23 with or without db-cAMP and RORγt inhibitor (RORc i) or Ahr inhibitor (Ahr i) overnight, suggesting that neither RORγt nor Ahr is likely needed for cAMP-enhanced IL-22 production. Data shown as means ± SEM are representative of four independent experiments. **P*<0.05, ***P*<0.01, ****P*<0.001 by one-way ANOVA.

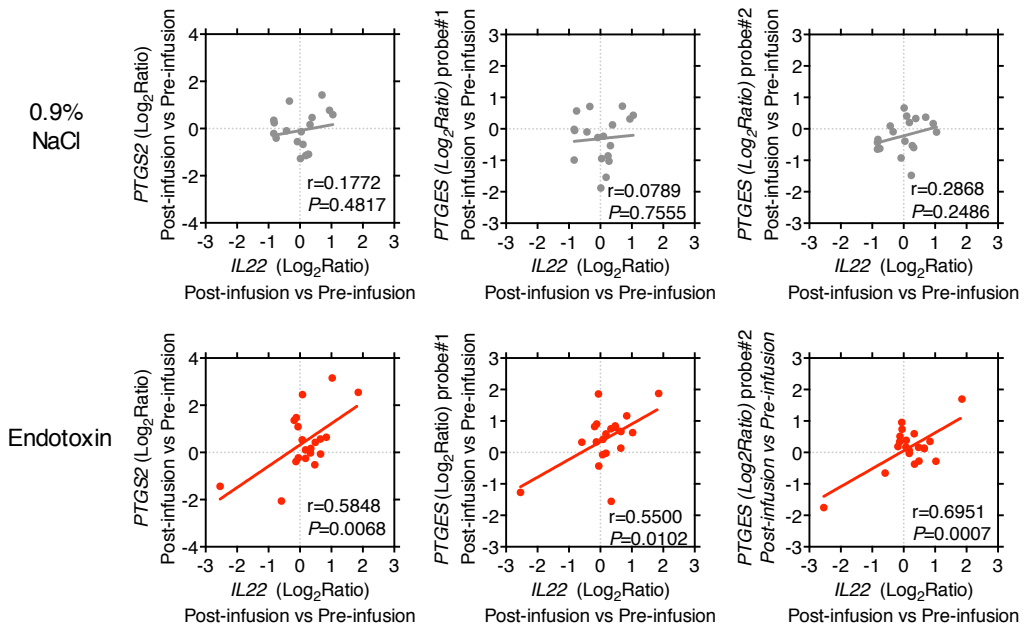

Fig. S13. Positive correlations between gene expression of *IL22* and PGE_2 synthases in response to endotoxin infusion in humans. Expression of *IL22* (encoding human IL-22), *PTGS2* (encoding human COX2) and *PTGES* (encoding human mPGES) genes in peripheral blood leukocytes obtained from healthy individuals before or after (2-24 h) infusion with endotoxin (CC-RE-Lot 2) or 0.9% sodium chloride ***(29)***. Raw data were retrieved from the Gene Expression Omnibus dataset GSE3284 ***(29)***. Data shown here were presented as the 'Log_2Ratio' of post-infusion (2, 4, 6, 9, 24 h) vs pre-infusion (0 h) of the endotoxin. Statistical analysis was calculated by the Pearson correlation coefficients (r).

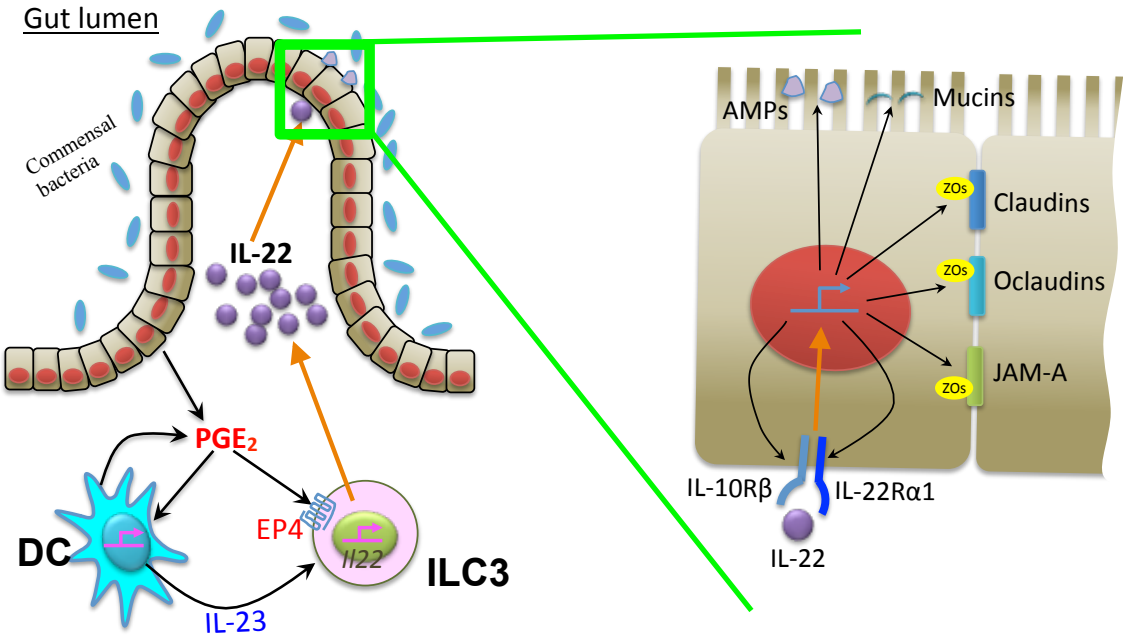

Fig. S14. A proposed model. PGE_2, produced by many types of cells such as macrophages, dendritic cells and gut epithelial cells, through the EP4 receptor contributes to maintaining ILC3 homeostasis. PGE_2 also works together with IL-23 to promote IL-22 production by ILC3s. The PGE_2/EP4/ILC3/IL-22 axis protects intestinal epithelial barrier function through amplification of IL-22-IL-22R signaling and promotion of production of antimicrobial peptides, mucus family proteins and tight junctions. This enables prevention of peripheral bacterial dissemination and subsequently contributes to control of systemic inflammation.

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