PNAS
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Supplementary Information for

Intestinal host defense outcome is dictated by PGE_2 production during efferocytosis of infected cells

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This PDF file includes:

www.pnas.org/cgi/doi/10.1073/pnas.1722016115

SUPPLEMENTAL METHODS

Mice. Microsomal prostaglandin E synthase-1 knockout mice (mPGES-1 KO mice - B6.129S6-Ptges2^{tm1Bhk}/J mouse strain) (1) were provided by Maziar Divangahi. Animals were housed and bred under specific pathogen-free conditions at the Institute of Biomedical Science (ICB), University of São Paulo (USP) according to Animal Ethics Committee of the ICB-USP. All animal experiments were carried out in accordance with the ethical guidelines and approved by the Institutional Animal Care and Committee from School of Pharmaceutical Sciences – UNESP.

Reagents. Naïve CD4$^+$ T cells were differentiated in CM or CM/Indo for 72 h in the presence of PGE_2 receptor agonists and antagonists, Butaprost (EP2 agonist; Cayman), AH6869 or PF04418948 (EP2 antagonist; Tocris), Cay 10598 (EP4 agonist; Cayman), L-161,982 (EP4 antagonist; Cayman), SC19220 or SC51089 (EP1 antagonist; Tocris), as well as Forskolin (adenylyl cyclase activator), 8-Bromo-cAMP (PKA activator), 8-CPT-2Me-cAMP (EPAC activator), PKI 14-22 amide-myristoylated (PKA inhibitor) and ESI-09 (EPAC inhibitor) (all Tocris); and neutralizing IL-1β antibody (BD) and IL-1Ra (Peprotech). The cells were stimulated as previously described, and the supernatants were collected for IL-17A quantification. The cells were analyzed for IL-17A expression by flow cytometry and for transcription factors related to Th17 cell differentiation. PGE_2 was also removed from CM using a Prostaglandin E_2 affinity column (Cayman). PGE_2 (10 nM; Cayman) was added exogenously to some cultures.

Flow cytometry analysis. After stimulation with 0.1 μg/mL phorbol 12-myristate 13-acetate (PMA; Sigma) and 0.5 μg/mL calcium ionophore (A23187) in the presence of brefeldin A (10 μg/mL; Sigma) for 4 h, the cells were labeled with fixable viability stain (BD), permeabilized, and incubated with anti-IL-17A-PECy7 or APC, anti-CD4-FITC or PE and anti-Foxp3-APC (BD and eBioscience). The cells were acquired by flow cytometry (FACS Canto - Becton & Dickinson, San Diego, CA, USA) and analyzed using the software FCS 4 Express Flow Cytometry (De Novo Software).

Phosflow assay. Approximately $5x10^5$ lymphocytes were cultivated in CM or CM/Indo or treated with EP4 agonist (Cay 10598) or antagonist (L-161,982), PKA activator (8-Bromo-cAMP), and EPAC activator (8-CPT-2Me-cAMP). After 15 min, the cells were harvested. The cells were fixed with Fixation Buffer (BD Cytofix) and incubated at 37°C for 12 min. The cells were washed again, treated with chilled Perm Buffer III (BD Phosflow), and incubated on ice for 30 min. The cells were stained with anti-phosphorylated STAT3-AlexaFluor 647 and anti-CD4-PE (BD).

ELISA. Supernatants were evaluated for the presence of cytokines and PGE_2. The minimum detectable concentrations were: 31.25 pg/mL for IL-6, IL-1β, and IL-10 (BD Pharmingen); 15.6 pg/mL for IFN-γ and TGF-β (R&D System); 7.8 pg/mL for PGE_2 (PGE_2 EIA Kit, Cayman Chemicals); 8 pg/mL for IL-23 (Biolegend); and 4 pg/mL for IL-17A (eBioscience). For the in vivo experiments, tissue was stored in PBS in the presence of protease inhibitor and indomethacin. Tissue homogenate was used for PGE_2 quantification. PGE_2 amounts were normalized by total protein concentration (Protein Assay kit, BioRad).

Quantitative real-time PCR (qPCR). RNA from $CD4^+$ T cell cultures or tissue homogenate was isolated using RNAspin Mini Kit (GE) according to the manufacturer's instructions (GE Healthcare) and reverse-transcribed into cDNA using the iScript cDNA Synthesis Kit (BioRad). Gene expression was determined by amplification with specific primers and quantification by SybrGreen (Life). The relative gene expression was calculated by the $2^{-\Delta Ct}$ method. For qPCR, a thermocycler AB 7500 (Applied Biosystems, Foster City, CA) was used. The sequences of the primers used are available in the SI Appendix, table S2.

qPCR Array. Naïve $CD4^+$ T cells were differentiated in the presence of CM, CM/Indo or CM/Ibup. Antibodies, anti-CD3 (4 μg/mL), anti-CD28 (2 μg/mL), anti-IL-2, anti-IL-4 and anti-IFN-γ (5 μg/mL) (BD) were added into the cultures. After 48 h, cells were harvest and RNA was extracted (RNeasy, Qiagen) followed by cDNA synthesis (RT2 microRNA First Strand Kit, Qiagen). Gene expression was evaluated by PCR array

(Th17 Response PCR Array, Qiagen). Plates were processed in a thermocycler AB 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA). Normalization of RNA expression was performed using SABiosciences Online PCR Array Data Analysis Web Portal. RNA expression was compared between cells differentiated in the presence of CM and CM/Indo or CM/Ibup. Fold change was calculated by $2^{-\Delta Ct}$ method. Data were analyzed using Qiagen-RT^2 Profile PCR Array analysis tool.

CFSE/Far-Red staining. The carboxyfluorescein succinimidyl ester stock solution (CellTrace™ CFSE) or Far-Red (Cell Proliferation Kit – Thermo Fisher Scientific) was diluted in pre-warmed PBS to the desired concentrations. Briefly, cells were incubated with CFSE or Far-Red at 37° for 15 min and then resuspended in a fresh pre-warmed medium for another 30 min and, then washed with PBS, according to the manufacturer's recommendation.

Immunofluorescence and confocal microscopy. $2.5x10^5$ BMDCs were labeled with Far-Red (1.5 μM) and cocultured for 4 h with $7.5x10^5$ ACs previously labeled with CFSE (1.5 μM). The cells were centrifuged at 1500 rpm for 10 min then removed, air dried and fixed with 4% formaldehyde solution for 20 min. Vectashield with DAPI (Vector Laboratories) was applied before coverslipping. Confocal microscopy was performed with wavelengths of 405nm for DAPI, 488nm for CFSE and 640nm for Far-Red excitation lines with an oil immersion objective (63x/1.4 numerical aperture). Imaging was performed using a Carl Zeiss LSM 800 inverted microscope equipped with Airyscan and images were acquired with ZEN BLUE 2.3 software.

Human monocyte isolation. Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized venous blood from healthy volunteers by density-gradient centrifugation using Ficoll (GE Healthcare). Briefly, blood was diluted in PBS to a volume of 30 mL and carefully transferred to 50-mL tube containing 10 mL of Ficoll, which was centrifuged at 2700 rpm for 20 min. Interface cells were collected and washed with PBS. Fresh $CD14^+$ monocytes were isolated using human CD14 Microbeads

(Miltenyi Biotech) according to the manufacturer's instruction. All procedures were performed after institutional ethical approval.

Monocyte-derived dendritic cell (MoDC) and generation of conditioned medium. Freshly isolated $CD14^+$ monocytes were cultured at a density of $1x10^6$/mL in complete medium (RPMI 1640 supplemented with 10% FBS). To generate MoDCs, recombinant human IL-4 (25 ng/mL; PeproTech), GM-CSF (50 ng/mL; PeproTech) and 25 μM of 2-β mercaptoethanol were added to the medium. On days 3 and 6, the RPMI medium was replaced with the same fresh complete medium. On day 7, the supernatant was removed, and the MoDCs were counted and resuspended at the desired concentration in RPMI medium. CM was generated by incubating MoDCs, in the presence or absence of 10 μM of indomethacin, with infected apoptotic cells (1:3 ratio). Supernatants for experimental controls were obtained from MoDCs left in resting conditions. The supernatant from each condition was collected for cytokines/PGE_2 quantification and used in human T cell assays.

Blockade of infected apoptotic cell (IAC) recognition and engulfment. IACs were incubated with Annexin V microbeads (Miltenyi Biotec) in RPMI medium supplemented with 2.5 mM of Ca^{2+}, according to the manufacturer's protocol. Briefly, IACs were incubated with the microbeads for 15 min at room temperature and then added to the BMDCs or MoDCs. IACs were also labeled with CFSE (1.5 μM). After 18 h of coculture with BMDCs or MoDCs in the presence or absence of Annexin V microbeads, the supernatant was collected for cytokine/PGE_2 quantification and used for naïve $CD4^+$ T cell differentiation assay. The percentage of efferocytosis ($CD11c^+CFSE^+$ cells) was analyzed by flow cytometry.

Naïve $CD4^+$ T cell differentiation under control conditions derived from BMDCs supernatants. BMDCs were treated for 20 min in the presence or absence 10 μM of COX inhibitor indomethacin (Sigma) or ibuprofen (Cayman). Conditioned medium (CM) was obtained from BMDCs cocultured with apoptotic *E. coli*-infected cells (IACs) at ratio 1:3 for 18 h. Supernatants for experimental controls were obtained from BMDCs

left in resting conditions in the absence of IACs, supernatants from IACs only, BMDCs cultured with uninfected apoptotic cells or BMDCs cultured with IACs in the presence of Annexin V microbeads (CM/Ann). The supernatant from each condition was collected for cytokines/PGE_2 quantification and used in naïve $CD4^+$ T cells differentiation assay. When indicated, PGE_2 was removed from CM by Prostaglandin E_2 affinity column (Cayman). After 72 h, the cells were stimulated for flow cytometry analysis of $CD4^+$IL-$17A^+$, $CD4^+Foxp3^+$ cells and, the supernatant was collected for IL-17A and IFN-γ quantification by ELISA.

Human lymphocyte isolation and mixed lymphocyte reaction. PBMCs isolated from heparinized venous blood from healthy volunteers were incubated at 37°C and 5% CO_2 during 2 h to allow monocytes adherence to the plate surface. Non-adherent cells in suspension were collected, and 10^5 lymphocytes were cocultured with 10^4 heterologous PBMCs in the presence of CM or CM/Indo supernatants for 72 h. Cells were labeled with fixable viability stain, anti-CD4, anti-IL-17A and assessed by flow cytometry

Enzyme-linked immunosorbent assay (ELISA) for CM derived from MoDC and human lymphocyte culture supernatants. Supernatants were evaluated for the presence of cytokines and PGE_2. The minimum detectable concentrations were: 2 pg/mL for IL-6 and IL-1β (all Thermo Fischer Scientific); 7.8 pg/mL for PGE_2 (PGE_2 EIA Kit, Cayman Chemicals); 156.3 pg/mL for TGF-β; 15 pg/mL for IL-23 and 5 pg/mL for IL-17A (all eBioscience).

Naïve $CD4^+$ T cell proliferation assay. Freshly isolated murine naive $CD4^+$ T cells were labeled with CFSE (1.5 μM) and cultured in CM or CM/Indo in the presence of anti-CD3 (4 μg/mL) and anti-CD28 (2 μg/mL). After 72 h, cells were labeled with anti-CD4 and assessed by flow cytometry.

Naïve $CD4^+$ T cell treatment. Murine naïve $CD4^+$ T cells were differentiated in CM in the presence of PGE_2 receptors antagonists: SC19220 or SC51089 (EP1 antagonist - 5 μM), AH6869 or PF04418948 (EP2 antagonist - 5 μM) (Tocris); L-161,982 (EP4

antagonist - 5 μM) (Cayman) and inhibitors of PKA and EPAC: H89 (PKA inhibitor – 5 μM) and ESI-09 (EPAC inhibitor – 5 μM) (all Tocris). Cells were incubated for 72 h and supernatants were collected for IL-17A quantification. Cells were stimulated as previously described and analyzed for IL-17A expression by flow cytometry and IL-17A quantification by ELISA.

***Citrobacter rodentium* infectious colitis model.** Female C57BL/6 wild-type (WT) (8-10 wk old) and, female and male mPGES-1 knockout mice (8-12 wk old) were orally infected with *Citrobacter rodentium* (ICC168) ($2x10^9$ CFU) in 200 μL of PBS. For control, animals received PBS alone. On 7^{th} or 8^{th} day of infection, the colonic tissue was collected, and tissue length and CFU counts were determined. Tissue homogenates were plated on LB solid agar containing nalidixic acid (NAL) (50 μg/mL - Sigma) for specific selection of NAL resistant *C. rodentium* bacteria in plates at 37°C for 24 h. To determine percentage of neutrophil and monocyte infiltration in the colon as well as IL-1R expression on $CD4^+$ T cells, WT animals were infected and treated intraperitoneally (i.p.) with vehicle (PBS+2% DMSO), indomethacin (5 mg/kg) on the 1^{st}, 3^{rd}, 5^{th} and 7^{th} days after infection or EP4 antagonist (L-161,982) (10 mg/kg) daily, during 7 days. Cells were obtained after colon digestion with collagenase D (Roche). Cells were labeled with fixable viability stain (BD), fixed and incubated with mouse anti-CD4-Pacific-Blue, anti-TCRβ-FITC, anti-IL-1R-APC, anti-Ly6G-PE and anti-Ly6C-PerCP-Cy5.5 (BD, eBioscience, Cayman, and Columbia Bioscience) and FcBlock (BD). To evaluate whether the efferocytosis contributes in the PGE_2 production in vivo, at day 6 of infection animals were treated intravenously (i.v.) with purified recombinant Annexin V (BD, 556416) (20 μg/mouse) and after 16 h colons were removed and stored in PBS in the presence of indomethacin. Tissue homogenate was used for PGE_2 quantification (ENZO) and gene expression analysis. PGE_2 amounts were normalized by total protein concentration (Protein Assay kit, Bio-Rad).

Lamina propria lymphocyte isolation. *C. rodentium*-infected mice were euthanized on day 8 after infection, and LPLs were isolated from colon according to Torchinsky et al. (2009) (2). First, the tissue was cut longitudinally and washed with PBS, processed in

small fragments, and incubated for 20 min at 37°C in PBS containing 2% FBS and 1 mM 1,4-Dithiothreitol (DTT; Sigma) with agitation. That procedure was repeated twice, and then a wash with PBS was performed. The tissue was incubated for 1 h at 37°C with RPMI 5% FBS containing 1.6 mg/mL collagenase D (Roche). After that, the tissue was homogenized using a 20G syringe and 70μm cell strainer. The wells and strainers were washed with RPMI medium to reduce cell loss. Cell pellets were resuspended in 15 mL tubes with 4 mL 44% Percoll solution and then transferred to 6 mL 66% Percoll solution. The mixture was then centrifuged at 2800 rpm for 20 min. Interface cells were collected using a plastic pipet, washed with PBS, and resuspended in IMDM with 10% FBS, PMA, ionomycin, and brefeldin A. The cells were stimulated for 4 h at 37°C before staining for analysis by flow cytometry.

Histopathological evaluation of colitis. Colon tissues were fixed in 10% buffered formalin, embedded in paraffin, stained with hematoxylin-eosin, and analyzed as previously described (3).

Statistical analysis. Data were presented as the mean ± SEM and analyzed by Prism 5.0 (GraphPad Software, San Diego, CA). For comparisons between experimental groups, one-way ANOVA was performed followed by Bonferroni or Tukey's multiple comparisons post-tests. Individual groups were compared using the Student's t test. Statistically significant differences were indicated by P values ≤ 0.05.

SUPPLEMENTARY FIGURES

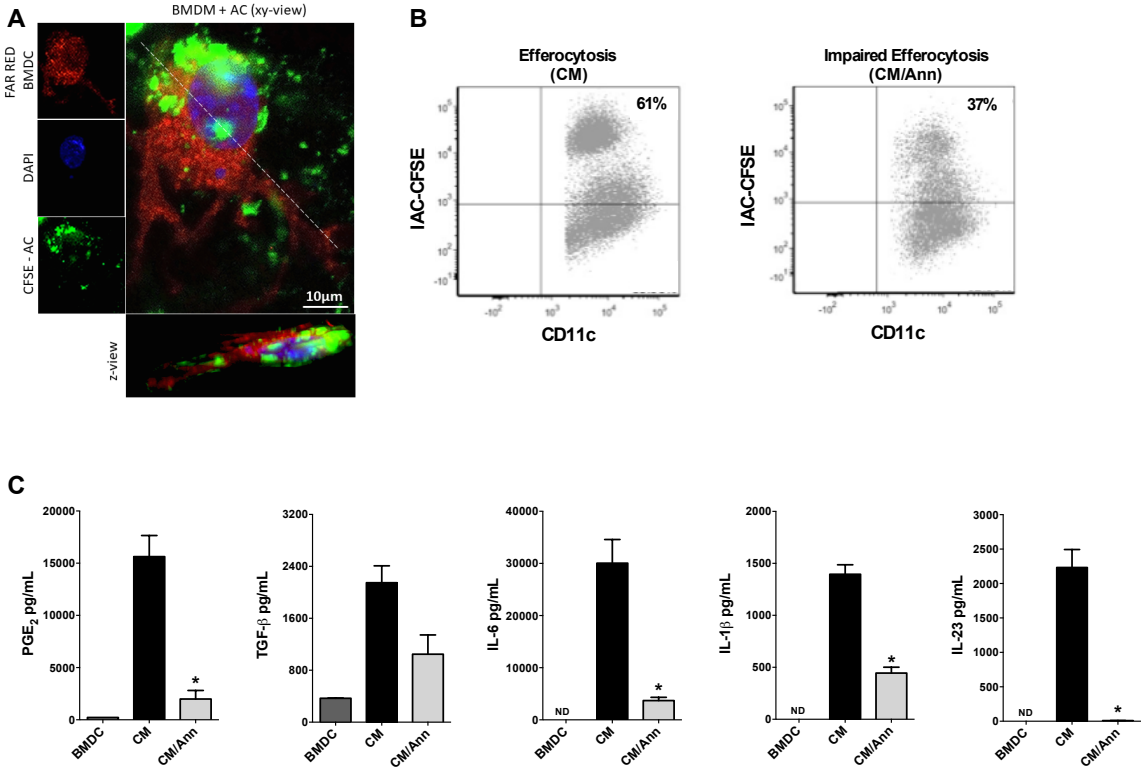

Fig. S1. Impaired efferocytosis of apoptotic *E. coli*-infected cells by BMDCs reduces PGE_2 production and cytokine release. Apoptotic *E. coli*-infected cells (IACs) labeled with CFSE were incubated in the presence or absence of Annexin V microbeads to impair efferocytosis, and then cocultured with BMDCs for 18 h. *(A)* Representative image of engulfment of apoptotic cell by BMDC. Confocal microscopy labeling for infected apoptotic cell (CFSE), BMDC (Far-Red) and DAPI obtained in microscopy oil immersion objective (63x/1.4 numerical aperture) using a Carl Zeiss LSM 800 inverted microscope equipped with Airyscan. Images were acquired with ZEN BLUE 2.3 software. *(B)* Flow cytometry determined efferocytosis index showed by representative dot plots of three independent experiments performed in triplicate. *(C)* ELISA measured the concentration of PGE_2 and cytokines in the supernatants derived from resting BMDC (BMDC), BMDCs cocultured with IACs (CM) or BMDCs cocultured with Annexin V microbeads-treated IACs (CM/Ann). Data represent mean ± SEM of two independent experiments performed in triplicate. ND= Not Detected. * $P < 0.05$ compared to CM.

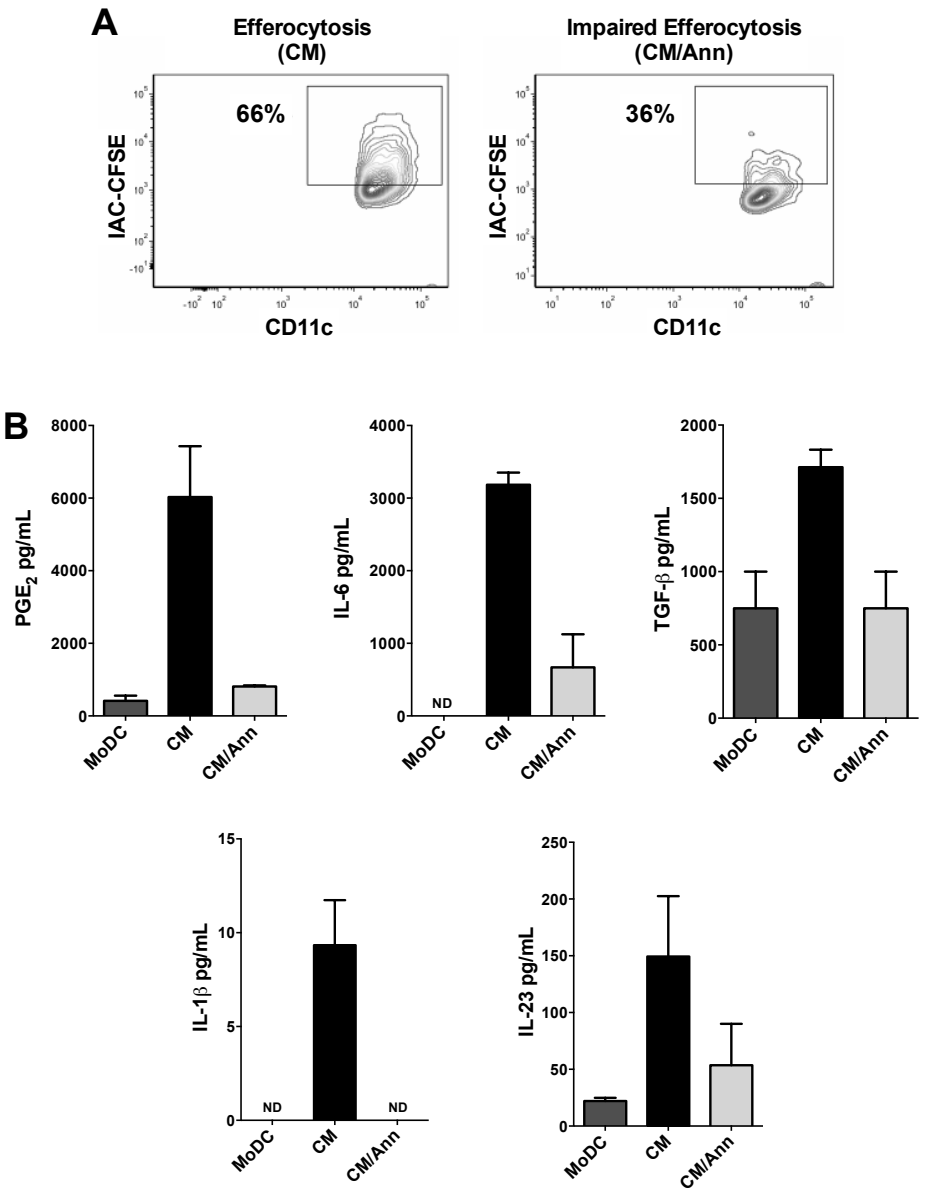

Fig. S2. Human efferocytosis of apoptotic *E. coli*-infected cells by MoDCs increases PGE_2 production and cytokine release. Apoptotic *E. coli*-infected cells labeled with CFSE were incubated or not with Annexin V microbeads and then cocultured with MoDCs for 18 h. *(A)* Efferocytosis was determined by flow cytometry and showed by representative dot plots of two independent experiments. *(B)* The concentration of PGE_2 and cytokines were measured by ELISA in the supernatants derived from resting MoDC (MoDC), MoDCs cocultured with IACs (CM) or MoDCs cocultured with Annexin V microbeads-treated IACs (CM/Ann). ND= Not Detected. Data represent mean ± SEM of two independent experiments performed in duplicate.

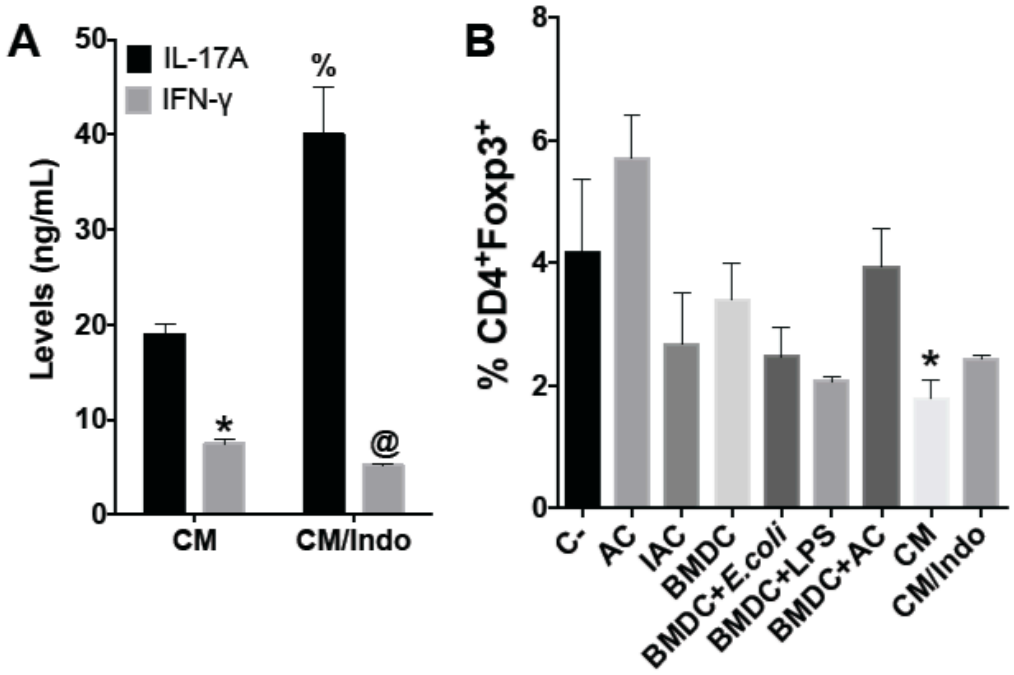

Fig. S3. Naïve $CD4^+$ T cell differentiation in the presence of supernatants from cocultures. *(A)* Naïve $CD4^+$ T cells activated with anti-CD3 and anti-CD28 were cultured in the presence of CM or CM/Indo for 72 h. CM and CM/Indo were generated by the efferocytosis of apoptotic *E. coli*-infected cells by untreated or indomethacin-treated BMDC, respectively. The levels of IL-17A and IFN-γ cytokines were detected by ELISA in the supernatants of T cell differentiation assays. Data represent mean ± SEM of three independent experiments performed in triplicate. * $P < 0.05$ compared to IL-17A of CM; @ $P < 0.05$ compared to IL-17A of CM/Indo; % $P < 0.05$ compared to IL-17A and IFN-γ of CM. *(B)* Naïve $CD4^+$ T cells were activated in the presence of supernatants from: apoptotic cells (AC), apoptotic *E. coli*-infected cells (IAC), resting BMDC (BMDC), BMDCs co-cultured with *E. coli* (ratio 1:3) (BMDC+*E. coli*), BMDCs treated with LPS (100 ng/mL) (BMDC+LPS), BMDCs co-cultured with non-infected apoptotic cells (ratio 1:3) (BMDC+AC), BMDCs co-cultured with apoptotic *E. coli*-infected cells (ratio 1:3) (CM) or indomethacin-treated BMDCs co-cultured with apoptotic *E. coli*-infected cells (ratio 1:3) (CM/Indo), or with only medium, as negative control (C-). After 72 h, cells were stimulated with PMA, ionomycin, brefeldin A and stained with anti-CD4 and anti-Foxp3 for flow cytometry analyses. The percentage of $CD4^+Foxp3^+$ T cells is demonstrated by bar graphs. Data represent mean ± SEM of at least three independent experiments performed in triplicate. * $P < 0.05$ compared to BMDC+AC.

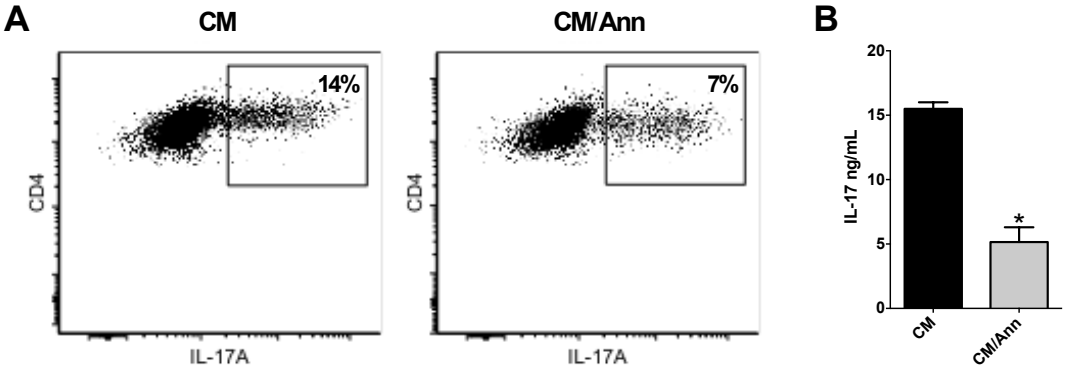

Fig. S4. Impaired efferocytosis of apoptotic *E. coli*-infected cells by BMDCs reduces Th17 cell differentiation. Naïve $CD4^+$ T cells were activated and differentiated with anti-CD3 and anti-CD28 in the presence of the conditioned media CM or CM/Ann for 72h. *(A)* The percentage of viable $CD4^+IL\text{-}17A^+$ T cells was determined by flow cytometry and showed by representative dot plots. *(B)* Levels of IL-17A released by lymphocytes were measured in the supernatant of cultures by ELISA. Data represent mean ± SEM of three independent experiments performed in triplicate. * $P < 0.05$ compared to CM.

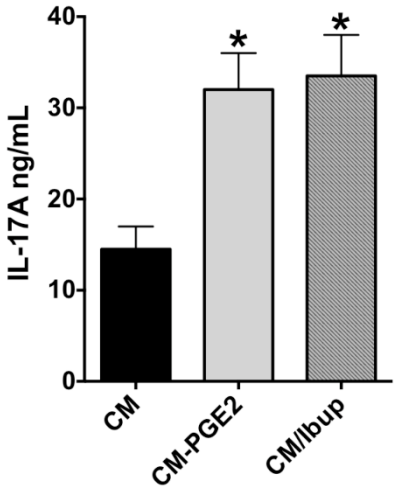

Fig. S5. PGE_2 derived from efferocytosis of apoptotic *E. coli*-infected cells by BMDCs inhibits Th17 cell differentiation. In the CM condition, BMDCs were untreated whereas, in CM/Ibup condition, BMDCs were previously treated with ibuprofen (10 μM) and then cocultured with apoptotic *E. coli*-infected cells at the ratio 1:3 for 18 h. In the CM-PGE_2 condition, PGE_2 was removed from CM using prostaglandin E2 affinity column. Naïve $CD4^+$ T cells activated with anti-CD3 and anti-CD28 were differentiated in the presence of CM, CM-PGE_2 or CM/Ibup for 72 h. The concentration of IL-17A released by lymphocytes was measured by ELISA. Data represent mean ± SEM of at least three independent experiments performed in triplicate. * $P < 0.05$ compared to CM condition.

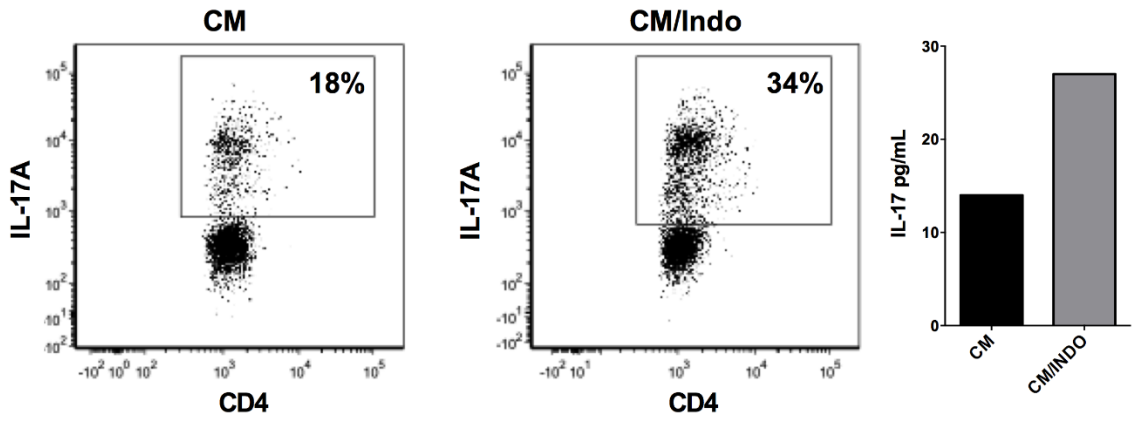

Fig. S6. PGE_2 produced by MoDCs during efferocytosis of apoptotic *E. coli*-infected cells impairs IL-17A expression by human lymphocytes. Human lymphocytes were isolated from peripheral blood and cocultured with heterologous PBMCs in the presence of CM or CM/Indo for 72 h. The percentage of viable $CD4^+IL\text{-}17A^+$ T cells was determined by flow cytometry and showed by representative dot plots. The levels of IL-17A released by lymphocytes were measured in the supernatant of cultures by ELISA. Data represent one experiment.

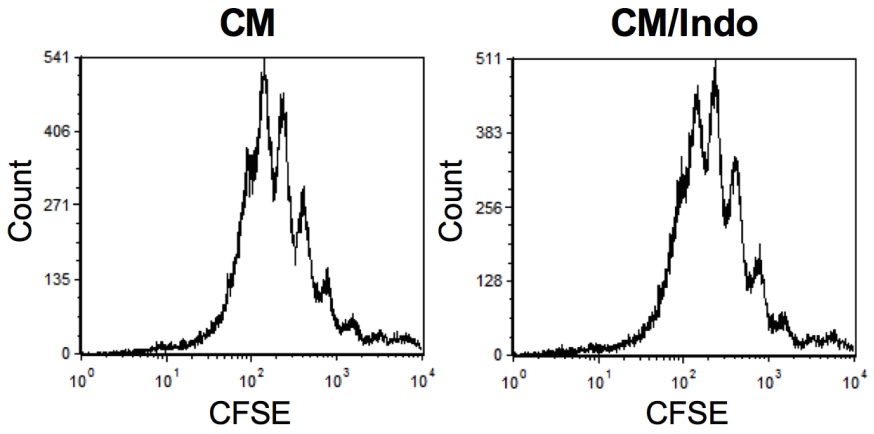

Fig. S7. PGE_2 in CM did not affect $CD4^+$ T cell proliferation. Naïve $CD4^+$ T cells previously labeled with CFSE were activated with anti-CD3 and anti-CD28 in the presence of conditioned medium for 72 h. Conditioned media were generated by the efferocytosis of apoptotic *E. coli*-infected cells by untreated BMDC (CM) or indomethacin-treated BMDC (CM/Indo). Proliferation was analyzed through CFSE dilution by flow cytometry.

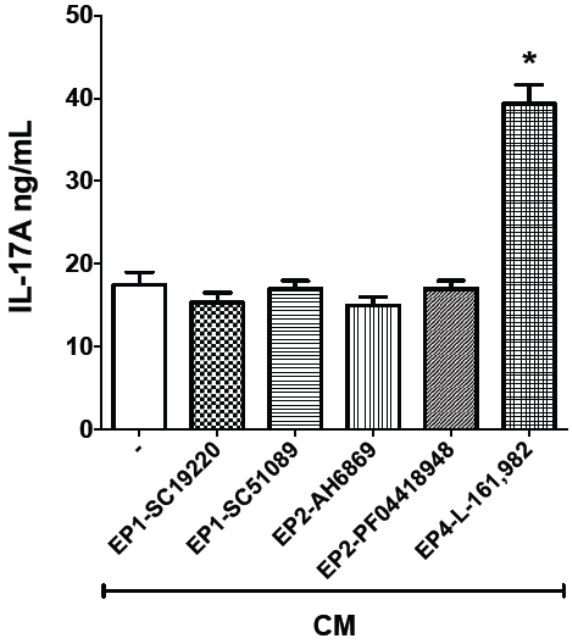

Fig. S8. EP1 and EP2 antagonists did not restore Th17 cell differentiation in the presence of CM. Naïve $CD4^+$ T cells were treated with EP1, EP2 or EP4 antagonists and differentiated in the presence of CM. After 72 h, the levels of IL-17A were detected by ELISA in the culture supernatants. Data represent mean ± SEM of three independent experiments performed in triplicate. * $P < 0.05$.

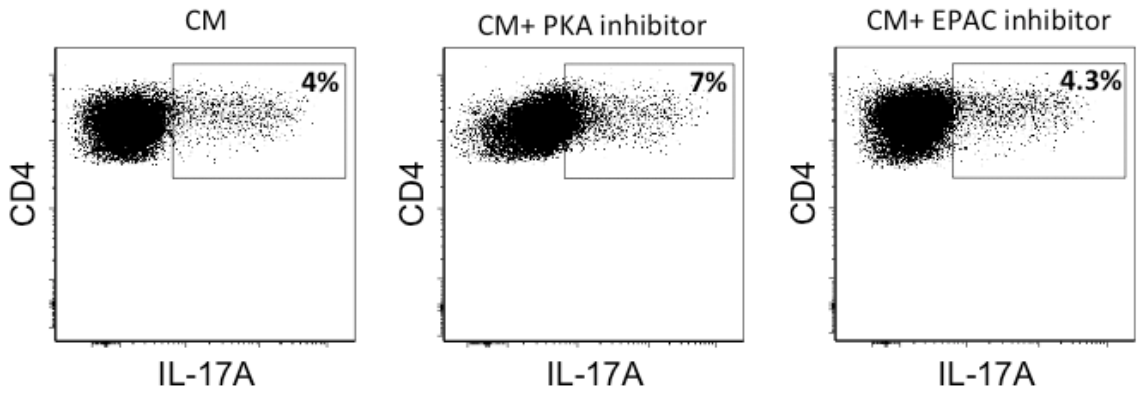

Fig. S9. PKA inhibitor improves Th17 cell differentiation in CM condition. Naïve $CD4^+$ T cells activated with anti-CD3 and anti-CD28 were cultured in CM condition in the presence of PKI 14-22 amide-myristoylated (PKA inhibitor) (5μM) or ESI-09 (EPAC inhibitor) (5μM). After 72 h, cells were stimulated with PMA, ionomycin and brefeldin A for 4 h and then washed and stained with fixable viability stain, anti-CD4 and anti-IL17A antibodies for flow cytometry analyses. Dot plots are representative of two independent experiments.

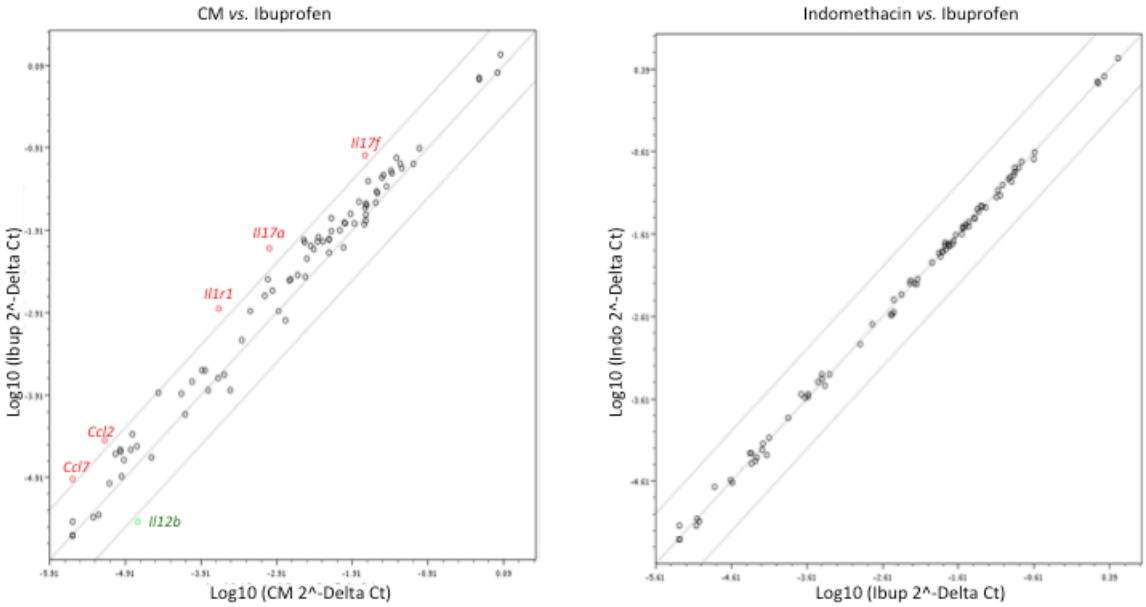

Fig. S10. Inhibition of PGE_2 synthesis during efferocytosis of apoptotic *E. coli*-infected cells by treating BMDCs with ibuprofen improves Th17-related gene expression. Naïve $CD4^+$ T cells were stimulated with anti-CD3 and anti-CD28 in the presence of supernatant from efferocytosis of apoptotic *E. coli*-infected cells by untreated BMDC (CM), indomethacin (CM/Indo) or ibuprofen (CM/Ibup) treated BMDC. After 48 h, RNA was extracted, and the expression of Th17 related genes was analyzed by qPCR array. Representative scatter plot of Th17 expressed genes by T cells differentiated in CM/Ibup (Y-axis) or CM (X-axis) condition (left) and by T cells differentiated in CM/Indo (Y-axis) or CM/Ibup (X-axis) condition (right). Fold change was calculated by $2^{-\Delta Ct}$. Genes 4-folder over or down-regulated are highlighted in the scatter plot.

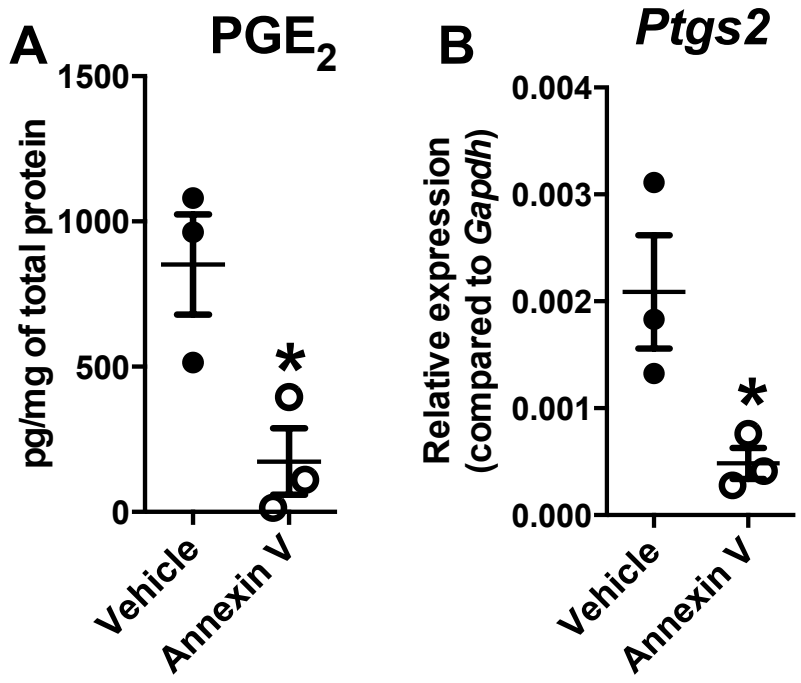

Fig. S11. Blockage of efferocytosis in vivo impairs PGE_2 production in the colonic tissue of *C. rodentium*-infected mice. WT mice were orally infected with *C. rodentium*. Six days post-infection animals were treated intravenously with recombinant Annexin V (20 μg/mouse) or vehicle (PBS). Colons were harvested after 16 h of treatment. *(A)* Levels of PGE_2 were measured in the colonic tissue and normalized by total proteins. *(B)* Gene expression of COX-2 (*Ptgs2*) was determined in the colons. Data represent mean ± SEM of one experiment. N= 3. * $P < 0.05$ compared to vehicle.

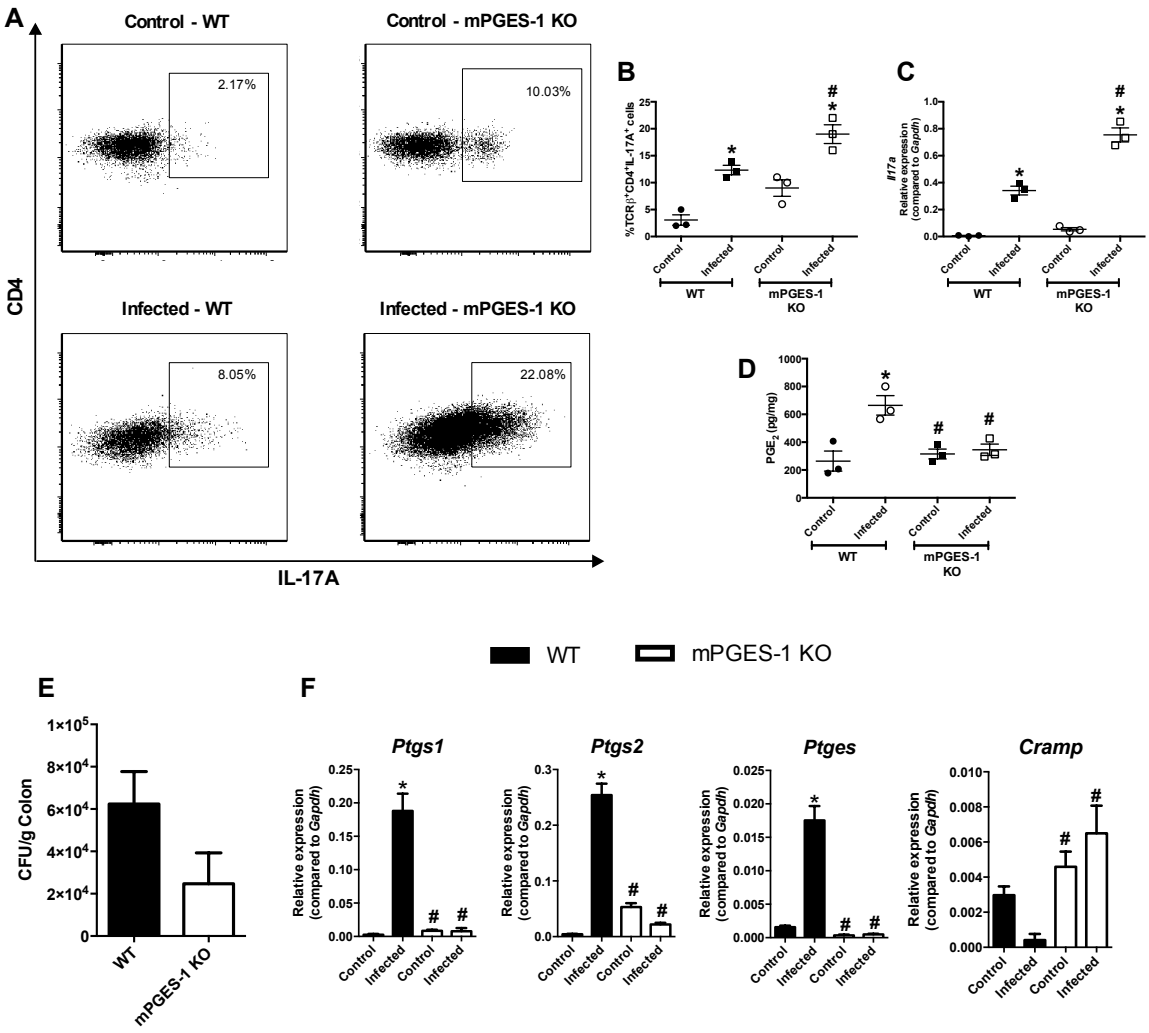

Fig. S12. In vivo deficiency of mPGES-1 improves Th17 cell population in the colonic tissue of *C. rodentium*-infected mice. WT and mPGES-1$^{-/-}$ mice were orally infected with *C. rodentium*. On the 7th day of infection colons were harvested. *(A, B)* The percentage of viable TCRβ^{+}CD4^{+}IL17A^{+} T cells in the colon were assessed by flow cytometry and demonstrated by representative dot plots and bar graph. *(C) Il17a* expression in the colonic tissue of WT and mPGES1$^{-/-}$ mice was determined by qPCR. *(D)* Levels of PGE_2 were measured in the colonic tissue and normalized by total proteins. *(E)* Colonic tissue homogenates were plated for CFU counting per gram of tissue. *(F)* The gene expression of COX-1 (*Ptgs1*), COX-2 (*Ptgs2*), mPGES-1 (*Ptges*) and cathelicidin (*Cramp*) was determined in the colons of WT or mPGES1$^{-/-}$ mice infected or not with *C. rodentium*. Data represent mean ± SEM of one experiment. N= 3. * $P < 0.05$ compared to Control (WT); # $P < 0.05$ compared to Infected (WT).

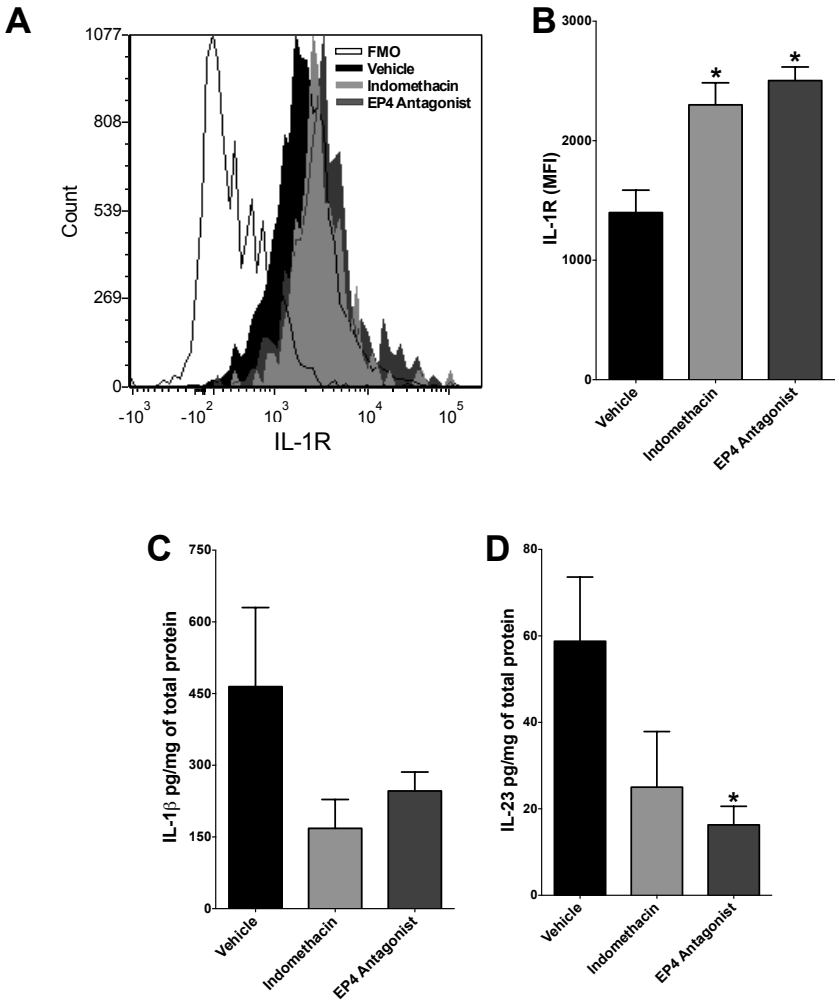

Fig. S13. In vivo inhibition of PGE_2 synthesis or EP4 signaling improves the expression of IL-1R on T cells and reduces the production of IL-1β and IL-23 in the colonic tissue of *C. rodentium*-infected mice. Mice were orally infected with *C. rodentium* and treated every other day with indomethacin (5 mg/kg), daily with L-161,982 (EP4 antagonist) (10 mg/kg) or vehicle during 7 days. On the 7th day of infection, colons were harvested. *(A, B)* MFI of IL-1R expression on T cells demonstrated by histogram and bar graphs. Cells were pre-gated on viable $CD4^+TCR\beta^+$ T cells. *(C)* Levels of IL-1β and *(D)* IL-23 were determined in the colonic tissue of *C. rodentium*-infected mice treated with vehicle, indomethacin or EP4 antagonist. Levels of cytokines were normalized by total protein. Data represent mean ± SEM of two independent experiments for Vehicle and Indomethacin groups and, a representative of two independent experiments for EP4 antagonist group. N=6-7. * $P < 0.05$ compared to Vehicle.

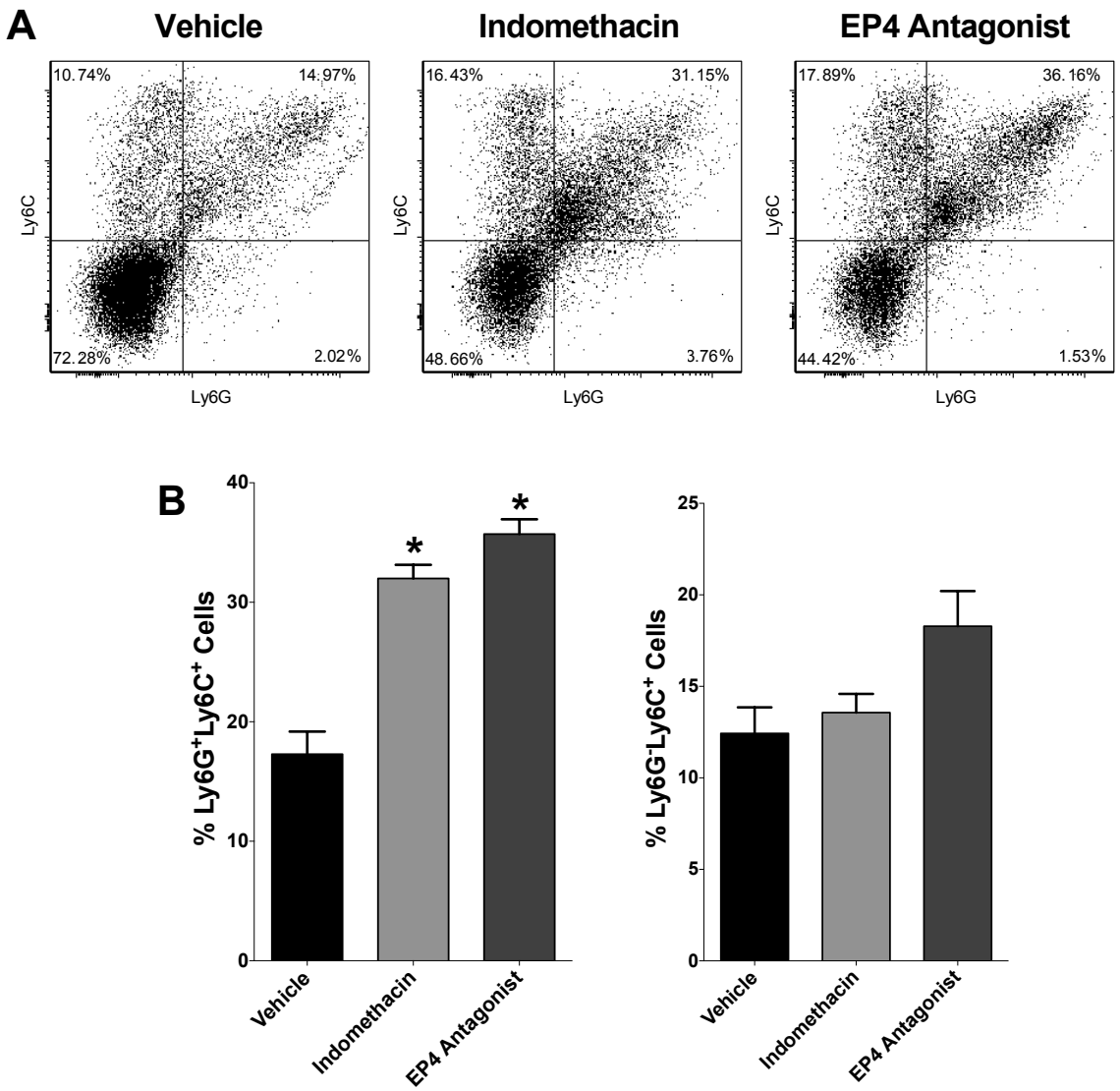

Fig. S14. In vivo inhibition of PGE_2 synthesis or EP4 signaling improves neutrophil recruitment to the colonic tissue of *C. rodentium*-infected mice. Mice were orally infected with *C. rodentium* and treated every other day with indomethacin (5 mg/kg) and daily with L-161,982 (EP4 antagonist) (10 mg/kg) or vehicle during 7 days. On the 7th day of infection, colons were harvested. *(A)* The percentage of $Ly6C^+$ and/or $Ly6G^+$ cells in the colon were assessed by flow cytometry and demonstrated by representative dot plots. Cells were pre-gated on viable cells. *(B)* The percentage of viable $Ly6G^+Ly6C^+$ cells or $Ly6G^-Ly6C^+$ cells were demonstrated by bar graphs. Data represent mean ± SEM of two independent experiments. N=7. * $P < 0.05$ compared to Vehicle.

Table S1. Fold change of 84 genes expressed by T cells differentiated in CM or CM/Indo condition. Naïve $CD4^+$ T cells were stimulated with anti-CD3 and anti-CD28 in the presence of CM or CM/Indo. CM and CM/Indo were generated by the efferocytosis of apoptotic *E. coli*-infected cells by untreated or indomethacin-treated BMDC (10 μM), respectively. After 48 h, RNA was extracted and the expression of Th17 cell differentiation related genes were measured by qPCR array. Table representing the comparison between Th17 expressed genes in T cells differentiated in CM/Indo and CM condition. Fold change was calculated by $2^{-\Delta Ct}$.

Genes	***Fold Change*** (CM/Indo *vs* CM)
Ccl1	1
Ccl2	4.79
Ccl20	-1.03
Ccl22	-1.23
Ccl7	4.93
Ccr2	1.34
Ccr4	2.38
Ccr6	1.33
Cd2	1.29
Cd28	1.63
Cd34	1
Cd4	1.44
Cd40lg	1.24
Cd8a	1.49
Cebpb	1.97
Clec7a	1.09
Csf2	-2.97
Csf3	2.44
Cx3cl1	1.87
Cxcl1	-1.29

Cxcl12	1
Cxcl2	1.67
Cxcl5	-1.05
Foxp3	2.22
Gata3	-1.27
Icam1	1.05
Icos	1.44
Ifng	-1.18
Il10	2.92
Il12b	-4.35
Il12rb1	1.77
Il12rb2	1.07
Il13	-1.85
Il15	-1.23
Il17a	7.41
Il17c	3.23
Il17d	1
Il17f	6.72
Il17ra	1.73
Il17rb	1.76
Il17rc	1.52
Il17re	3.72
Il18	1.18
Il1b	1.42
Il1r1	7.79
Il2	2.01
Il21	1.73
Il22	1.66
Il23a	-1.14
Il23r	3.23

Il25	1
Il27	1.95
Il3	1.13
Il4	1.88
Il5	2.01
Il6	-1.3
Il6ra	2.93
Il7r	1.51
Il9	2.88
Irf4	1.74
Isg20	-1.3
Jak1	1.15
Jak2	1.16
Mmp3	1
Mmp9	-1.09
Myd88	1.68
Nfatc2	2.08
Nfkb1	1.22
Rora	2.33
Rorc	3.6
Runx1	2.55
S1pr1	1.57
Socs1	1.59
Socs3	1.59
Stat3	1.78
Stat4	1.01
Stat5a	2.34
Stat6	1.88
Syk	-1.04
Tbx21	1.45

Tgfb1	1.4
Tlr4	-1.23
Tnf	1.71
Traf6	1.68
Actb	1.36
B2m	1
Gapdh	1.44
Gusb	1.49
Hsp90ab1	1.44

Table S2. Primer sequences used in qPCR.

Il17a	F:AGGCAGCAGCGATCATCC	R:GTGGAACGGTTGAGGTAGTC
Il17f	F:TTGATGCAGCCTGAGTGTCT	R:AATTCCAGAACCGCTCCAGT
Il1r1	F:ACCGTGAACACAAATGG	R:GAGGCACCATGAGACAAATG
Ccl2	F:AACTCTCACTGAAGCCAGCTCT	R:CGTTAACTGCATCTGGCTGA
Ccl7	F:CAATGCATCCACATGCTGC	R:CTTCCCAGGGACACCGAC
Ptgs1	F:AGGAGATGGCTGCTGAGTTGG	R:AATCTGACTTTCTGAGTTGCC
Ptgs2	F:GGGCCCTTCCTCCCGTAGCA	R:TGAGCCTTGGGGGTCAGGGA
Ptges	F: CAGAGCCCACCGCAACGACA	R: CCCAGGTAGGCCACGGTGTG
Cramp	F:CTTCAACCAGCAGTCCCTAGACA	R:TCCAGGTCCAGGAGACGGTA
Mbd1	F:CCAGATGGAGCCAGGTGTTG	R:AGCTGGAGCGGAGACAGAATCC
Mbd2	F:AAGTATTGGATACGAAGCAG	R:TGGCAGAAGGAGGACAAATG
Mbd3	F:GCATTGGCAACACTCGTCAGA	R:CGGGATCTTGGTCTTCTCTA
Mbd4	F:GCAGCCTTTACCCAAATTATC	R:ACAATTGCCAATCTGTCGAA
Reg3g	F:TCAGGTGCAAGGTGAAGTTG	R:GGCCACTGTTACCACTGCTT
Gapdh	F:AACTTTGGCATTGTGGAAGG	R:ACACATTGGGGGTAGGAACA
Il22	F:GCTCAGCTCCTGTCACATCA	R:CACTGTCTCCTTCAGCCTTCT
Ep1	F:CTCCTTGCGGCATTAGTGTG	R:TGCGGTCTTTCGGAATCGT
Ep2	F:CGTTATCCTCAACCTCATTCGC	R:TCCGTCTCCTCTGCCATCG
Ep3	F:TTGCTGGCTCTGGTGGTGAC	R:GCTGGACTGCGAGACGGC
Ep4	F:TGACCCAAGCAGACACCACCT	R:TCCCACTAACCTCATCCACCAA

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