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Supplementary information

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Inulin-gel-based oral immunotherapy remodels the small intestinal microbiome and suppresses food allergy

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Supplementary Methods

Characterization of inulin with different DPs. Inulin samples were characterized via ^{1}hydrogen-nuclear magnetic resonance ($H^{1}NMR$) and matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF). Inulin samples were obtained from Sigma-Aldrich (DP23), Now Foods (DP10) and Swanson (DP7). Without specific annotation, inulin or inulin gel means inulin from chicory with a DP value of 23 (Sigma-Aldrich).

***In vivo* sensitization, oral immunotherapy and challenges.** For alum/PE model, C3H/HeJ mice (5-6 weeks old females from Jackson Laboratory) were i.p. sensitized with PE (50 μg per dose, Stallergenes Greer Ltd.) and alum (2 mg per dose) in 150 μL PBS on days 0, 14 and 28. Naïve mice, included as a healthy control, were not sensitized. From day 43, the sensitized mice were orally administered with PBS, PE, inulin/PE or inulin gel/PE for 25 days (4 times per week, inulin: 55 mg per dose, PE: 1 mg per dose). From day 49, all mice were orally challenged with 200 mg peanut powder every other day for 5 times. For CT (List Biological Laboratories)/PE model, C3H/HeJ mice (5-6 weeks old females from Jackson Laboratory) were orally sensitized with PBS (sham-sensitization, naïve group), or PE (6 mg per dose) and CT (10 μg per dose) in 200 μL PBS on days 0, 1, 2, 7, 14, 21, and 28. From day 36, these mice were orally administered with PBS, PE, inulin gel or inulin gel/PE for 29 days (4 times per week, inulin: 45 mg per dose, PE: 1.2 mg per dose). Naïve mice were orally administered with PBS as a control. On day 73, mice were i.p. injected with 125 μg PE and the body temperature was recorded. For alum/casein model, BALB/c mice (5-6 weeks old females from Jackson Laboratory) were i.p. sensitized with PBS (sham-sensitization, naïve group), or casein (50 μg per dose) and alum (1 mg per dose) in 150 μL PBS on days 0 and 14. From day 29, mice were orally administered with PBS, casein, inulin gel, inulin/casein or inulin gel/casein for 25 days (3 times in 4 days, inulin: 55 mg per dose, casein: 1 mg per dose). From day 65, the mice were orally challenged with 300 μL cow's milk (Maple Hill® organic zero sugar milk, Kroger in USA) on 6 alternating days in 2 weeks.

Assessment of hypersensitivity reactions and diarrhea. Anaphylactic scores were evaluated by visually monitoring the mice for up to 60 minutes after i.p. or i.g. challenge of allergen[1]. Anaphylactic symptoms were scored: 0, no symptoms; 1, scratching and rubbing around the nose and head; 2, puffiness around the eyes and mouth, pilar erection, reduced activity, and/or decreased activity with increased respiratory rate; 3, wheezing, labored respiration, and cyanosis around the mouth and the tail; 4, no activity after prodding or tremor and convulsion; and 5, death. In alum/OVA model, mice demonstrating profuse liquid stool during 1 h after i.g. challenge were recorded as diarrhea-positive. The clinical diarrhea scores were assessed via visually observation: 0, normal; 1, soft; 2, running; 3, liquid; 4, bloody.

Allergen-specific IgE detection. For OVA allergic mice, serum samples were collected one day before the 1st i.g. challenge or after the 3rd i.g. challenge with 50 mg of OVA. For casein allergic mice, serum samples were collected on days 70 and 77. Allergen-specific antibody in serum was detected via enzyme linked immunosorbent assay (ELISA) method. Briefly, the 96-well ELISA plate was coated with 1μg of OVA or casein per well and incubated overnight at 4°C. The plate was washed four times and blocked with 1% BSA in PBS buffer for 2 h. After washing, serum samples were diluted and incubated in 96-well plate for 1 h. After repeated washing, horseradish peroxidase (HRP)-conjugated goat anti-mouse IgE (1:4000 dilution, SouthernBiotech) was added to each well and incubated for 1 h. The plate was thoroughly washed and 100 μL of 3,3',5,5'-tetramethylbenzidine (TMB) solution was added to each well. 15 min later, the stop solution (1 M H_2SO_4) was added and the optical density (OD) value at 450 nm was measured via a microplate reader.

MMCP-1 detection. Serum samples were collected in 45-60 min after the 6th i.g. challenge. MMCP-1 was detected following the instruction of the commercial Mouse MCPT 1 (mMCP 1) ELISA Ready SET Go! Kit (ebioscience).

Cytokine expression. 24 h after the 6th i.g. challenge, the spleens from various groups were obtained aseptically. The splenocytes were cultured in 96-well plates (1×10^6 cells per well) and re-stimulated with OVA (250 μg/mL). After 72 h of incubation, the supernatant was obtained and the cytokines were measured via LEGENDplex™ MU Th Cytokine Panel (12-plex) w/ VbP V03 (Biolegend, USA) using CytoFLEX flow cytometer (Beckman) and the data were analyzed by LEGENDplex™ Clound-Based Software.

SCFAs analysis. The ileal contents were collected on day 49, weighted and stored in -80°C until ready for analysis. On the day of extraction, samples were transferred to bead-filled homogenization tubes, 300 μL of 70% isopropanol were added and then homogenized via Precellys + Cyrolys Evolution Homogenizer at the following settings: 4°C temperature, 6000 rpm speed, 4 x 20 s cycle, 120 s pause time. An additional 700 μL of 70% isopropanol was added to the homogenate, vortexed and then transferred into a fresh Eppendorf tube. 300 μL of the homogenate was used to determine the dry weight (DW) of the ileal matter and the remaining homogenates were stored in -80°C until ready for further analysis. 2 mg DW/mL of samples were prepared from the stored homogenates, then centrifuged at 17,000g, 4°C for 15 min, and the supernatant was collected. 9-Point external standards were used for quantification with concentration ranging from 0 to 62.5 μM of acetic acid, propionic acid and butyric acid. Prior to derivatization, 50 μL of the internal standard solution containing 100 μg/mL of acetic acid (2-13C, 99%), 100 μg/mL of sodium propionate (D5, 98%) and

500 μg/mL of butyric acid (D7, 98%) was added to 50 μL samples and external calibration standards. This was followed by the addition of 20 μL of 200 mM 3-nitrophenylhydrazine hydrochloride (3-NPH·HCl) in 50% acetonitrile/water, followed by adding 20 μL of 120 mM N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC·HCl) (6% pyridine) in 50% acetonitrile/water. After incubation at 40°C for 30 min, the solutions were cool down on ice, and 200 μL of mobile phase A (0.1% formic acid in water) was used to quench the reaction. Samples went through 0.2 μm filters to remove potential particulates before running on LC/MS. Filtrates were analyzed with SCIEX Triple Quad™ 7500 LC-MS/MS- QTRAP system coupled with ExionLC™ system. The MS method was set up using the following parameters: GS1 = 45 psi, GS2 = 70 psi, spray voltage = 1600 V, Temperature = 450°C, settling time = 15 ms and pause time = 3 ms**.** Analytes were separated using Phenomenex's kinetex, 2.6 μM F5 100 A (150 x 2.1 mm) equipped with SecurityGuard ULTRA Cartridge. The MRMs and some parameters were obtained from the literature[2-4]. Mobile phase A is 0.1% formic acid in water while mobile phase B is 0.1% formic acid in acetonitrile. Each run took 4 minutes with gradient set up as follows: 0 min 10% B, 0.3 min 20% B, 2.5 min 23% B, 2.6 min 100% B, 3.0 min 100% B, 3.1 min 10% B and finally 4 min 10% B. Column temperature was set at 60°C. The flowrate was 0.25 mL/min and 2 μL of filtrate from each sample were injected per run. Peaks were extracted using SCIEX OS (version 2.1.6.59781) and further processing was performed in excel.

Tregs-induction *in vitro*. For the inulin gel/allergen-induced Tregs study, BMDCs (4×10^4 cells/well in 96-well plate) were incubated with inulin, inulin gel (3.67 mg/well) in the presence or absence of OVA protein (40 nM) or OVA-II peptide (1 nM). 24 h later, the cells were washed for five times with cell culture medium. Splenocytes from OT-II mice were obtained and $CD4^+$ T cells were isolated via EasySep™ Mouse $CD4^+$ T Cell Isolation Kit (Stemcell Technologies, USA) and labeled with carboxyfluorescein succinimidyl ester (CFSE). $CD4^+$ T cells were added (10^5 cells/well) and co-cultured with these BMDCs in 96-well plate in the presence of IL-2 (100 U/mL, PeproTech) and TGF-β (10 ng/mL, R&D Systems). After 5 days, cells were collected and stained with Treg cells kit for flow cytometry. For the metabolite-induced Tregs study, flat 96-well plate was coated with Hamster IgG (1 μg/well) overnight. Pan $CD4^+$ T cells isolated from the transgenic Foxp3(GFP) reporter mice (7-week-old) were seeded with 4×10^4 cells/well in presence of anti-CD3 (1 μg/mL), anti-CD28 (1 μg/mL), IL-2 (5 ng/mL), and TGF-β (1 ng/mL). Subsequently, metabolites with various concentrations (10 μM, 50 μM) were added to 96-well plate and cultured for 3 days. The expression of Foxp3 was detected using GFP as a biomarker via flow cytometry.

Th1- and Th2-cell induction *in vitro*. Flat 96-well plate was coated with anti-CD3/28 antibodies (1 μg/mL) overnight, and washed once with PBS for cell culture. Splenocytes from WT C57BL/6 mice (7-weeks old

females from Jackson Laboratory) were obtained and $CD4^+$ T cells were isolated via EasySep™ Mouse $CD4^+$ T Cell Isolation Kit. Naïve $CD4^+$ T cells were added to 96-well plate (10^5 cells/well) in the presence of IL-2 (100 U/mL). The following antibodies were also added to the medium for Th1- or Th2-inducing: 10 ng/mL IL-12 p40 (PeproTech) and 10 μg/mL anti-IL-4 antibody (clone 11B11, BioXCell) for Th1 induction; 10 ng/mL IL-4 (PeproTech) and 10 μg/mL anti-IFN-γ (clone XMG1.2; BioXcell) antibody for Th2 induction. Subsequently, metabolites with various concentrations (10 μM, 50 μM) were added to 96-well plate and cultured for 5 days. Cells were stimulated with PMA and ionomycin for 2 h, then blocked with brefeldin A for another 2 h. Cells were stained with fixable efluor 780 dye, PE-Cy7-CD4 Rat anti-Mouse, APC-Foxp3 Monoclonal Antibody, PE-IL-4 Rat anti-Mouse, FITC-IL-10 Rat anti-Mouse, BV421-anti-mouse IFN-γ for flow cytometry.

BMDCs and metabolites co-culture assay. BMDCs were seeded in 12 well plates at a density of 0.5 million cells in each well. BMDCs were incubated with 1 μg/mL LPS, LPS plus dexamethasone (10 nM), LPS plus IL-10/TGF-β (20 ng/mL), LPS plus 5-thymidylic acid (100 μM), LPS plus acetic acid (100 μM), LPS plus propionic acid (100 μM), LPS plus butyric acid (100 μM), LPS plus uridine (100 μM), LPS plus 2'-deoxyguanosine (100 μM), or LPS plus guanosine (100 μM). 24 h later, the cells were harvested and stained with the following antibodies for flow cytometry analysis: Fixable efluor450, BV785-Anti-CD11c Armenian Hamster Monoclonal Antibody (Clone: N418, BioLegend), PerCP/Cy5.5-anti-mouse/human CD11b (Clone: M1/70, BioLegend), FITC-anti-mouse I-A/I-E (Clone: M5/114.15.2, BioLegend), AF594-anti-mouse CD80 (Clone: B7-1), and APC-anti-mouse CD86 (Clone: B7-2, BioLegend).

Gut microbiota depletion by antibiotic cocktail. BALB/c mice (5-6 weeks old females from Jackson Laboratory) were sensitized by alum/OVA. From day 29, these mice were orally administered with inulin gel (55 mg per dose)/OVA (1 mg per dose). Some mice were further orally administered with 100 μL of DI water containing ampicillin (1 mg/mL), metronidazole (1 mg/mL), streptomycin (0.5 mg/mL), and vancomycin (0.5 mg/mL) to deplete the gut microbiota for 20 days. From day 49, the mice were i.g. challenged with 50 mg of OVA protein for 4 times and the anaphylactic scores, diarrhea score, and the body weight at the 4^{th} challenge were recorded.

Immune cells depletion by antibodies. Alum/OVA-sensitized BALB/c mice (5-6 weeks old females from Jackson Laboratory) received OIT treatment for 3 weeks. α-CD25 (clone PC-61.5.3; BioXcell) was injected i.p. in some mice on days 0, 3, 7, 10, 14 (100 μg per dose). α-IL-10 (clone JES5-2A5; BioXcell), or α-IFN-γ (clone XMG1.2; BioXcell) was i.p. injected in some mice on days 0, 3, 7, 10, 14, 17, 20 (100 μg per dose, designated as OIT phase depletion setting). Meanwhile, α-IL-10, or α-IFN-γ was i.p. injected in some other

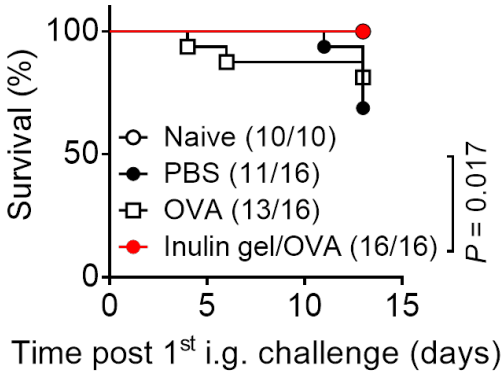
mice on days 14, 17, 20, 24, 27 (100 μg per dose, designated as challenge phase depletion setting). Rat IgG1 isotype control (clone HRPN; BioXcell) was injected on days 0, 3, 7, 10, 14, 17, 20, 24, 27 (100 μg per dose) as a control group. From day 21, the mice were i.g. challenged with 50 mg of OVA protein on 6 alternating days in 2 weeks. The body weight and temperature change at the 6^{th} challenge were recorded.

CBC and biochemical analyses. Mice were treated as in **Fig. 1b**. On day 48, serum and blood samples were collected and the *In-Vivo* Animal Core of the University of Michigan tested the complete blood count (including WBC+RG1:W1, neutrophils, lymphocyte, monocyte, eosinophil, basophils, red blood cell counts (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), platelet count (PLT), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC)), and the biochemical analysis (including alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), and creatinine (CREA)).

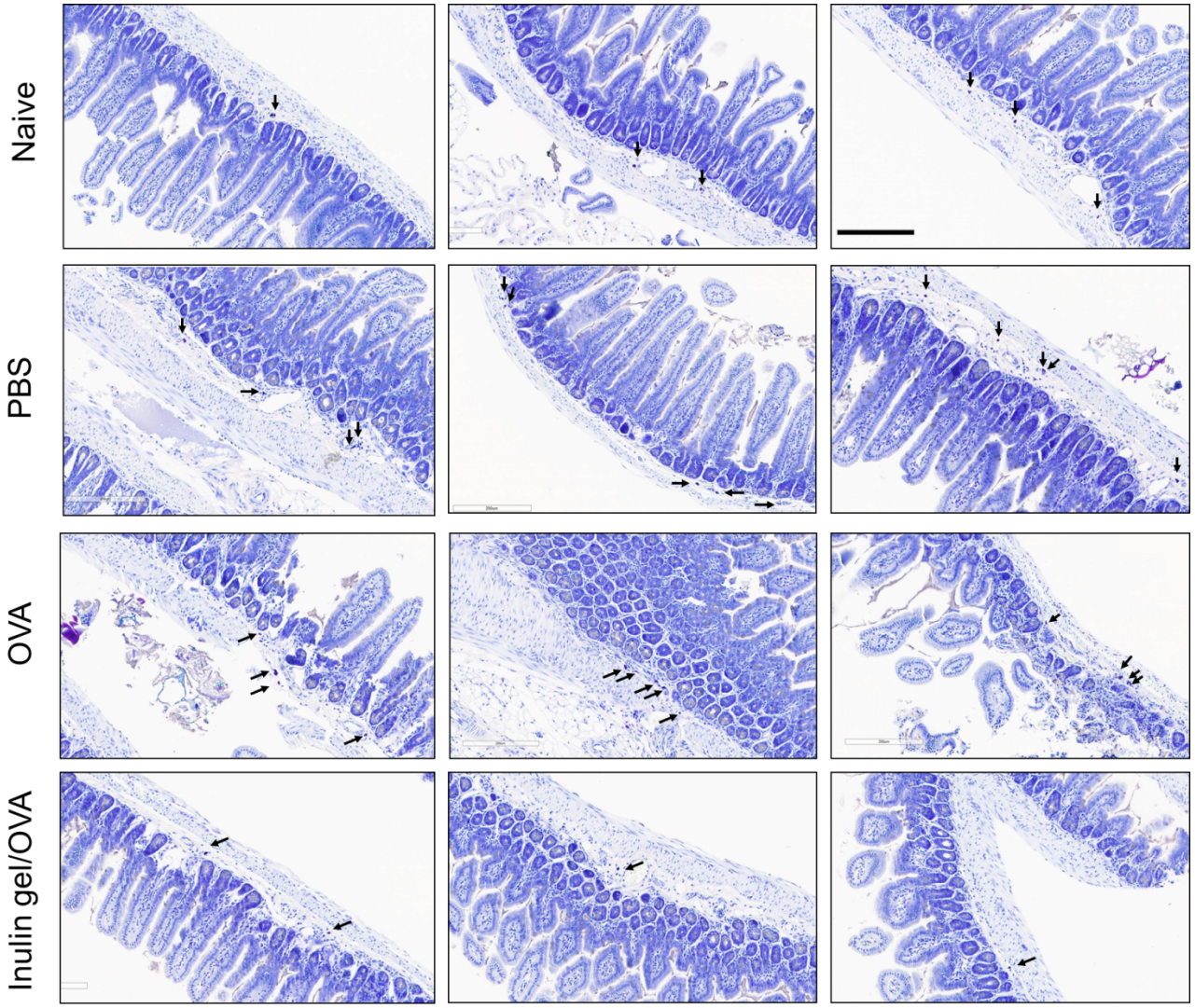
Eosinophil measurement in the lung and colon. For the food allergy model, alum/OVA-sensitized BALB/c mice received OIT treatment, repeated i.g. challenges as described in "*In vivo* sensitization, oral immunotherapy and challenges" section. On day 48, the fecal samples were collected to measure the cholic acid level. On day 61, the lungs were isolated and processed as described below. For the naive mice model, 6-week-old C57BL/6 mice were either fed with inulin chow diet (D15092907, Research Diets, **Supplementary Table 1**) without additional treatment, or with normal chow diet without inulin supplement (D12450J, Research Diets, **Supplementary Table 1**). Mice fed with normal diet received oral gavage of PBS, soluble inulin (55 mg/dose, 3 times per 4 days), or inulin gel (55 mg/dose, 3 times per 4 days). On day 13, the fecal samples, serum and caecal content were collected, and the concentrations of bile acids (CA, TCA, ursodeoxycholic acid (UDCA), DCA, ω-MCA, CDCA) were measured in the Pharmacokinetics and Mass Spectrometry Core of University of Michigan. The colon tissues were processed in the same way as the small intestines except that the digestion was prolonged to 2 hours. The lung tissues were collected, minced, and agitated in digestion buffer as previously described[5]. The digestion buffer contained RPMI-1640, 5% fetal bovine serum, 1500 U/mL type-1 collagenase, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 1 μg/mL DNase. The cells were then passed through a 70 μm filter, resuspended in 40% Percoll-RPMI medium, added on top of 67% Percoll-PBS solution and centrifuged for 20 min at 650 g. The leukocytes were collected at the interface, washed with FACS buffer and stained with the following antibodies: BV421-CD45 Rat Monoclonal Antibody (Clone: 30-F11, BioLegend), Pacific Blue-CD19 Rat Monoclonal Antibody (Clone: 6D5, BioLegend), BV510-anti-mouse I-A/I-E antibody (Clone: M5/114.15.2, BioLegend), BV711-Anti-Ly6C Rat Monoclonal Antibody (Clone: HK1.4, BioLegend), BV785-Anti-CD11c Armenian Hamster Monoclonal Antibody (Clone: N418, BioLegend), FITC-anti-

mouse/human CD11b (Clone: M1/70, BioLegend), PE-Anti-CD64 Mouse Monoclonal Antibody (Clone: X54-5/7.1, BioLegend), PE/Cy7-anti-mouse Ly6G (Clone: 1A8, BioLegend), AF647-Siglec-F Rat anti-mouse (Clone: E50 2440, BD), AF700-anti-CD3 Rat Monoclonal Antibody (Clone: 17A2, BioLegend). The cells were analyzed by Cytek Aurora in the Flow Cytometry Core at the University of Michigan.

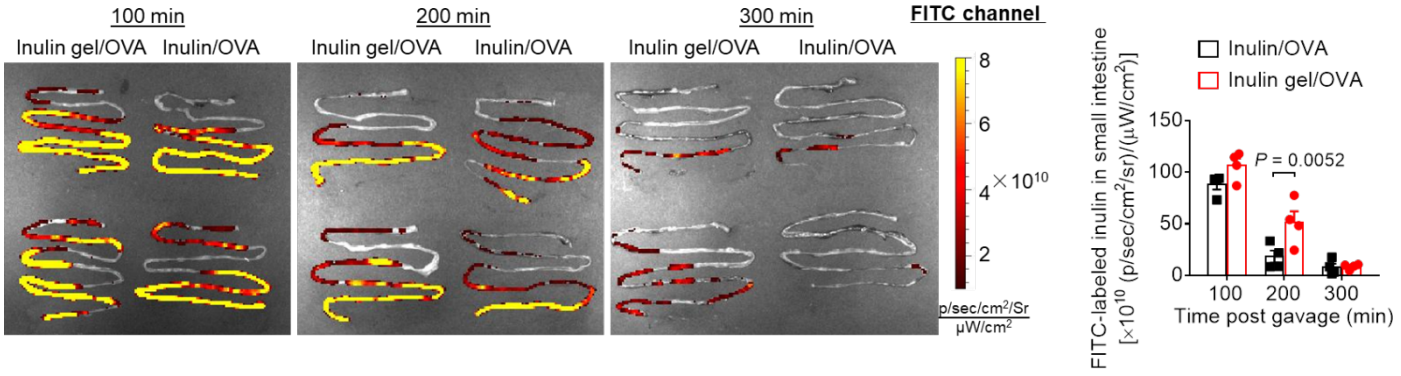
Supplementary Figures



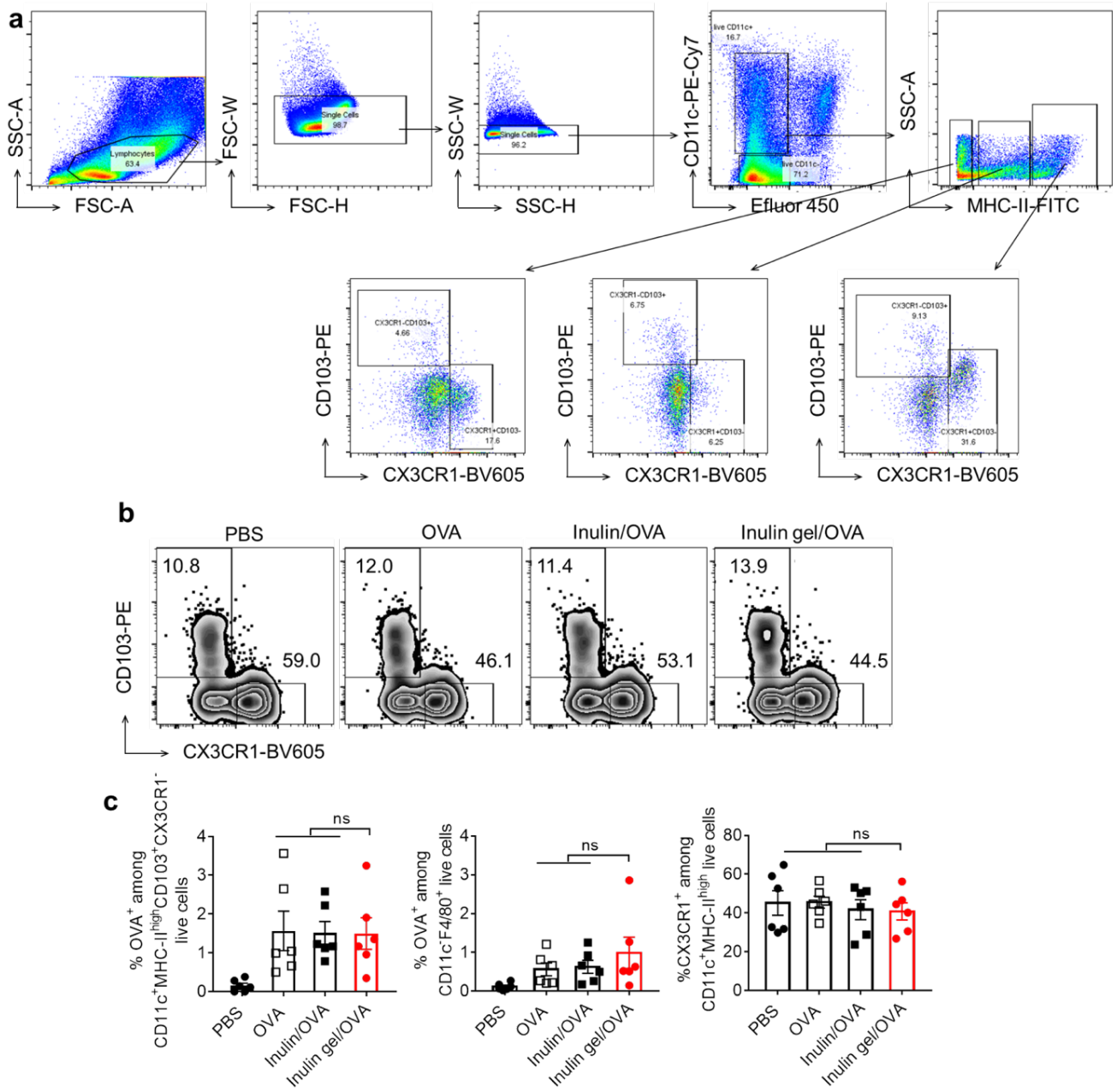
Supplementary Fig. 1. BALB/c mice were treated as in **Fig. 1b**. Shown are the overall Kaplan–Meier survival curves and number of survivors in each group during the i.g. challenges of 50 mg of OVA protein. The day of 1st i.g. challenge was day 0. Results are pooled from two independent experiments (n = 10 for Naïve or 16 for all other groups). Data were analysed using Log-rank (Mantel-Cox) test.



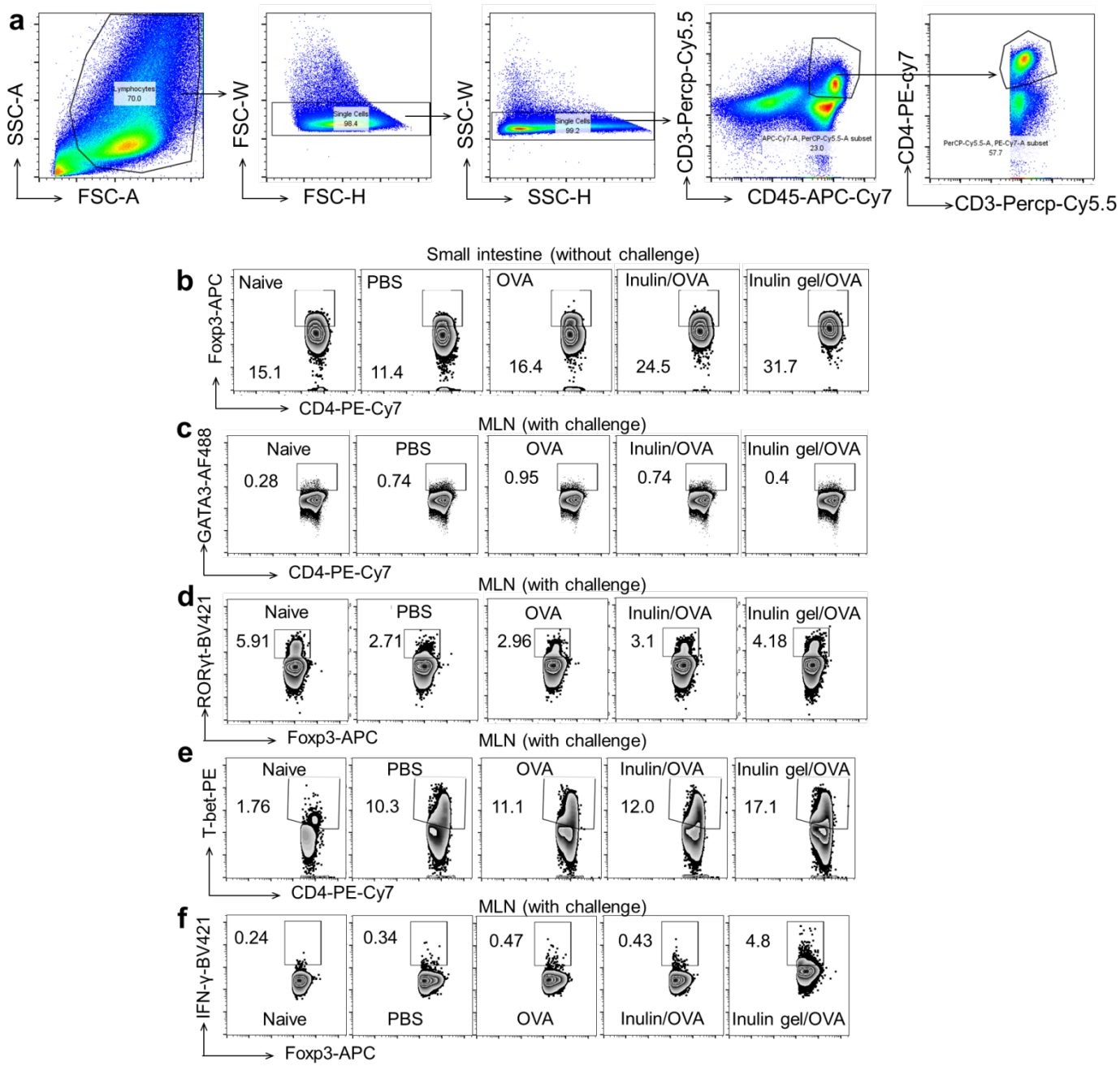
Supplementary Fig. 2. Shown are additional representative images of Toluidine blue staining of jejunal mast cells (arrows) per group for **Fig. 1k**. Scale bar: 200 μm.



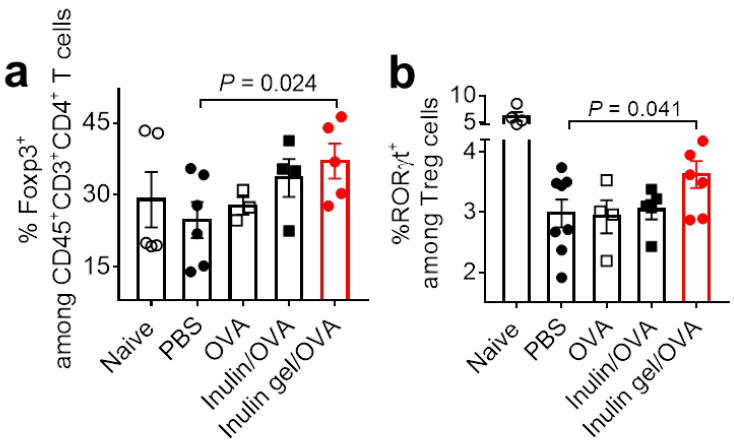
Supplementary Fig. 3. Alum/OVA-sensitized mice were orally administered with FITC-labeled inulin gel/Texas Red-labeled OVA or FITC-labeled inulin/Texas Red-labeled OVA (inulin: 55 mg per dose, OVA: 1 mg per dose). Shown are the representative images and the total fluorescence intensity of FITC-labeled inulin in the small intestine under FITC channel. Exposure time: 0.5 s. Data represent mean ± s.e.m. (n = 4 biologically independent samples). Data were analysed by two-way ANOVA with Bonferroni's multiple comparisons test.



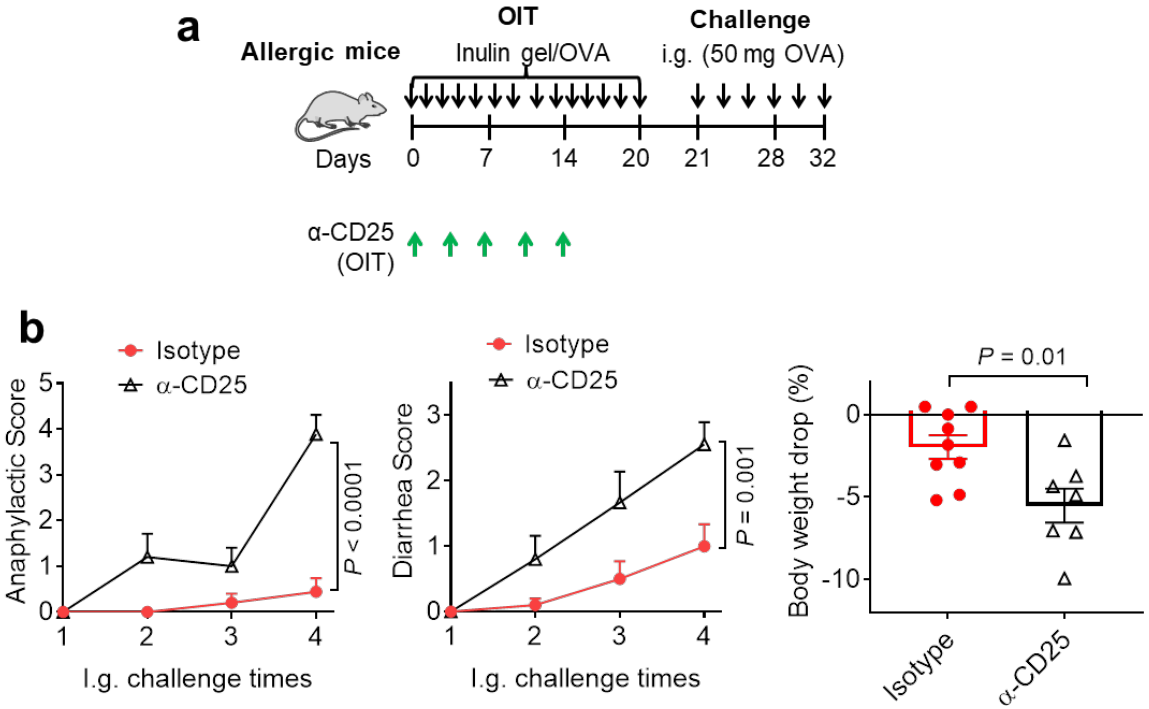
Supplementary Fig. 4. Alum/OVA-sensitized BALB/c mice received OIT treatment and then were orally administered with inulin gel/OVA-AF647. Mice were euthanized at 3 h after oral gavage, and the small intestine was processed for flow cytometry analysis. **a**, Shown is the gating strategy for analyzing different types of APCs. **b**, The representative flow cytometry plots with the percentage of $CD103^{+}CX3CR1^{-}$DC cells are shown. **c**, OVA-AF647 uptake in $CD11c^{+}MHC\text{-}II^{high}CD103^{+}CX3CR1^{-}$ cells, $CD11c^{-}F4/80^{+}$ macrophages and the frequency of $CX3CR1^{+}CD11c^{+}MHC\text{-}II^{high}$ cells are shown. Data represent mean ± s.e.m. (n = 6 biologically independent samples). Data were analysed by one-way ANOVA with Bonferroni's multiple comparisons test.



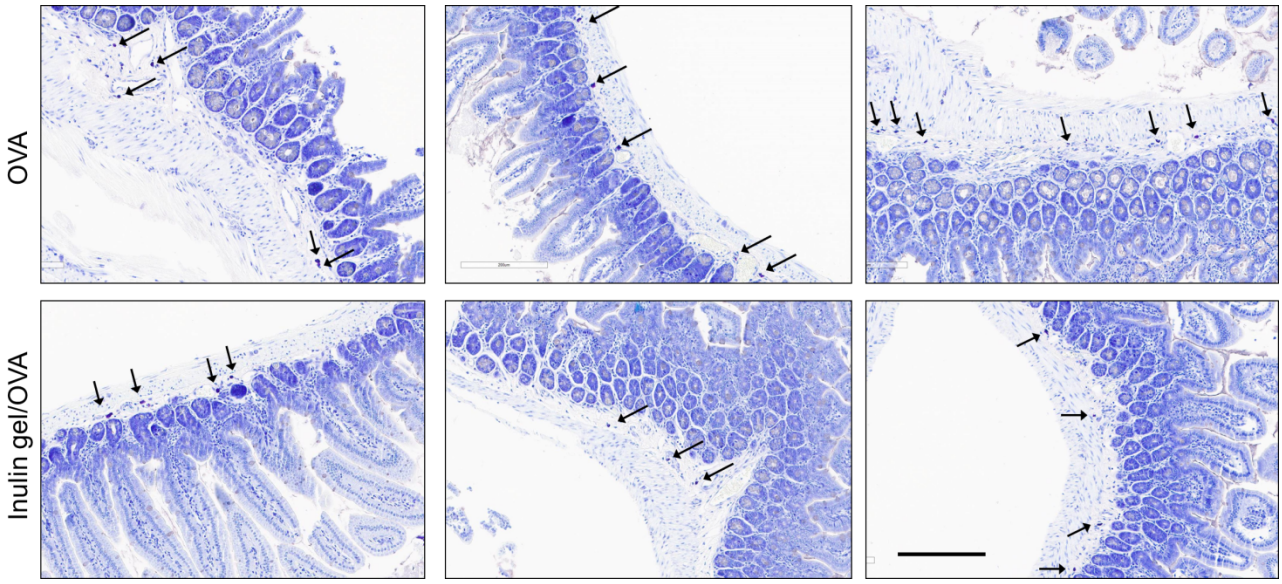
Supplementary Fig. 5. Alum/OVA-sensitized BALB/c mice were treated as in **Fig. 1b**. MLN and the small intestines were processed for flow cytometric analysis. **a**, Gating strategy. **b-f**, Representative scatter plots with percentages of Tregs in SI-LP before i.g. challenge of OVA, and $GATA3^{+}CD4^{+}$ T cells, $ROR\gamma t^{+}$ Tregs, $T\text{-}bet^{+}CD4^{+}$ cells, and IFN-γ^{+} Tregs after i.g. challenges. **b**, **c**, **e** were gated on $CD4^{+}$ T cells, while **d**, **f** were gated on $Foxp3^{+}CD4^{+}$ T cells.



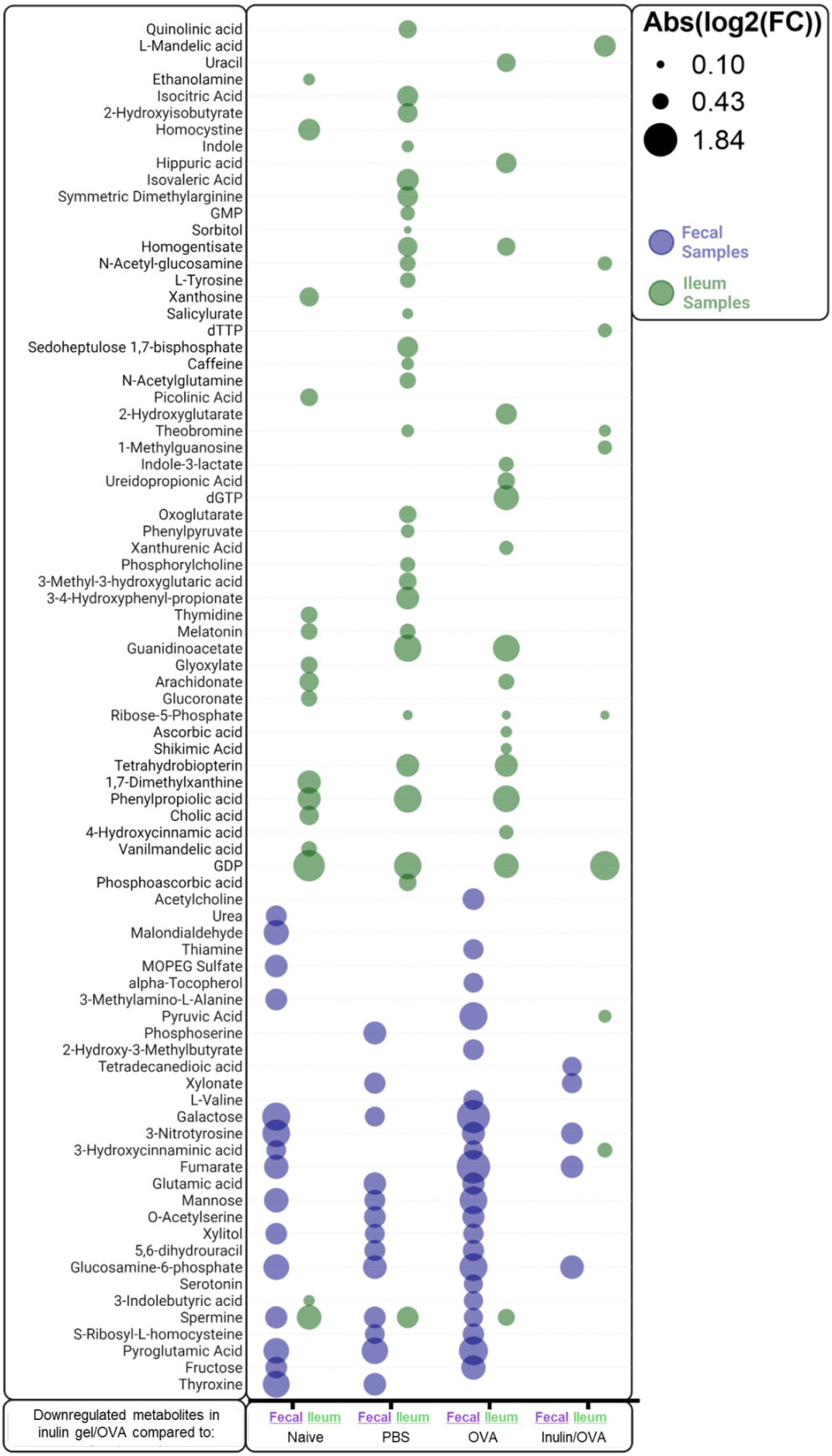
Supplementary Fig. 6. Alum/OVA-sensitized BALB/c mice were treated as shown in **Fig. 1b**. Shown are the frequencies of Foxp3$^+$CD4$^+$ Tregs in SI-LP (**a**), and RORγt$^+$ Tregs in MLN (**b**) on day 57. Data represent the mean ± s.e.m. (n = 3 for OVA, 4 for Inulin/OVA, 5 for Naive and Inulin gel/OVA, or 6 for PBS (**a**), n = 4 for Naive and OVA, 5 for Inulin/OVA, 7 for Inulin gel/OVA, or 8 for PBS (**b**) biologically independent samples). Data were analysed by one-way ANOVA with Bonferroni's multiple comparisons test.



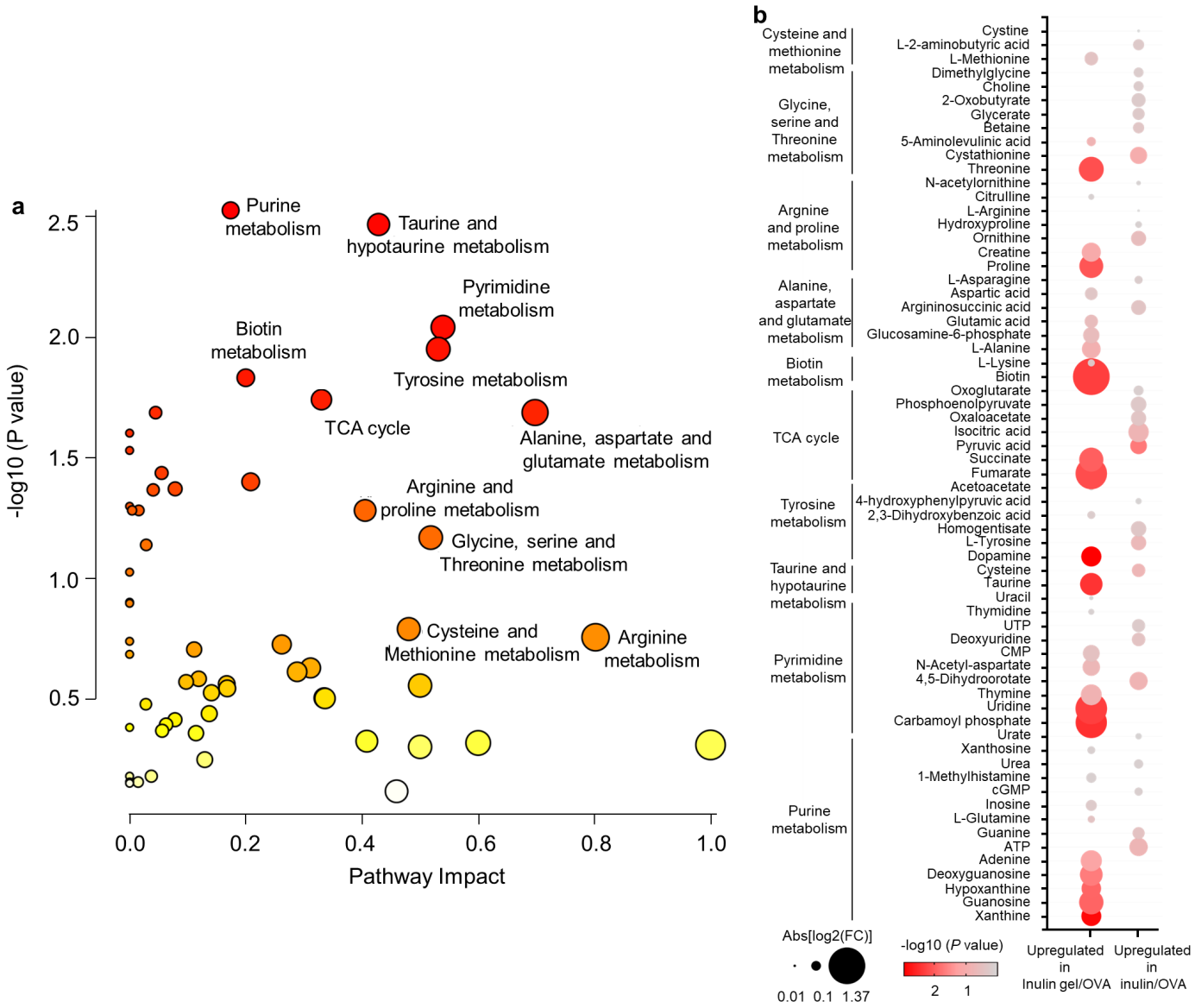
Supplementary Fig. 7. Alum/OVA-sensitized BALB/c mice were treated as shown in (**a**). 100 μg of antibody against Tregs (α-CD25), or isotype control was administered i.p. as indicated. Shown are the changes in anaphylactic score, diarrhea score during the consecutive i.g. challenge, and the body weight drop after the 4th i.g. challenge (**b**). Data represent the mean ± s.e.m. (n = 9 for Isotype or 7 for α-CD25 in Body weight drop dataset, n = 10 for all other datasets). Data were analysed by two-way ANOVA with Bonferroni's multiple comparisons test, or unpaired, two-sided Student's t-test.



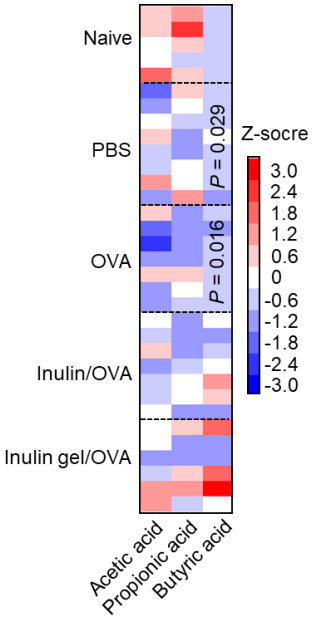
Supplementary Fig. 8. Shown are additional representative images of Toluidine blue staining of jejunal mast cells (arrows) per group for **Fig. 4f**. Scale bar: 200 µm.



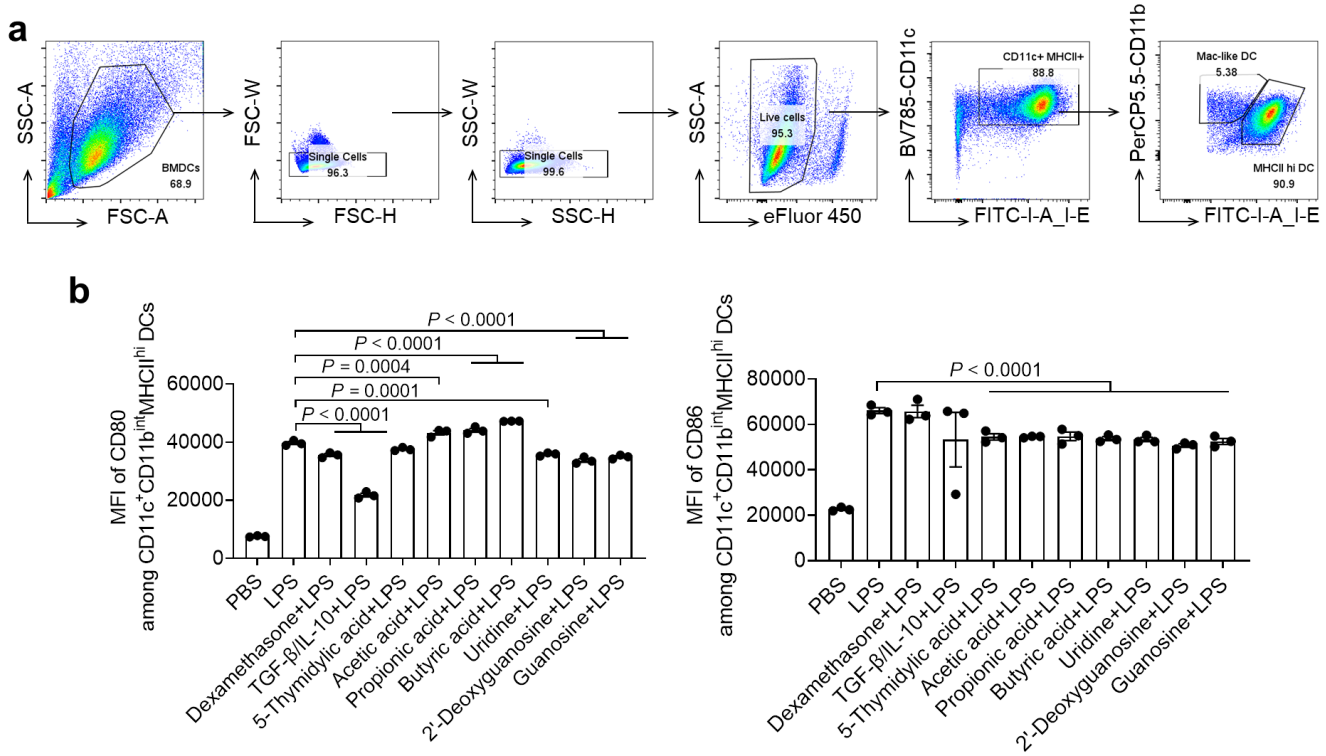
Supplementary Fig. 9. Alum/OVA-sensitized BALB/c mice were treated as in **Fig. 1b**. Shown are the fold changes of metabolites from the ileal and fecal samples, whose *P*-value is less than 0.05. The size of the circle denotes the fold change of downregulated metabolites in inulin gel/OVA group, compared with naïve, PBS, OVA, and inulin/OVA groups. The *P*-values are unadjusted and are based on a two-sided t-test statistics.



Supplementary Fig. 10. Alum/OVA-sensitized BALB/c mice were treated as in **Fig. 1b**. Shown are the pathway analysis between inulin gel/OVA and inulin/OVA groups, Pathway score are computed based on over representation analysis (ORA) in Metaboanalyst® (Version 5.0), *P* values were computed using right-tailed Fisher's exact test (**a**) and markedly changed metabolites in different metabolic pathways (**b**) in the ileal contents on day 49. FC: fold change. The *P*-values are unadjusted and are based on a two-sided t-test statistics.



Supplementary Fig. 11. Alum/OVA-sensitized BALB/c mice were treated as in **Fig. 1b**. Shown is the heatmap of the normalized Z-score values of SCFAs in the ileum. Data represent the mean ± s.e.m. (n = 5 for Naïve, 8 for PBS, 7 for OVA and Inulin/OVA, or 6 for Inulin gel/OVA biologically independent samples). P-values refer to statistical significance in comparison to the inulin gel/OVA group. Data were analysed by one-way ANOVA with Bonferroni's multiple comparisons test.



Supplementary Fig. 12. BMDCs were incubated with various metabolites in the presence of LPS for 24 h. Dexamethasone and TGF-β plus IL-10 were used as controls. Shown are (**a**) the gating strategy for $CD11c^{+}CD11b^{int}MHC\text{-}II^{hi}$ DCs and (**b**) the MFI values of CD80 and CD86 among $CD11c^{+}CD11b^{int}MHC\text{-}II^{hi}$ DCs. Data represent the mean ± s.e.m. (n = 3 biologically independent samples). ***$P < 0.001$, ****$P < 0.0001$. Data were analysed by one-way ANOVA with Bonferroni's multiple comparisons test.

Pairwise Spearman
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
OTU
Metabolites
Prevotellaceae_unclassified 0090
Muribaculum 0018
Muribaculaceae_unclassified 0008
Desulfovibrio 0023
Helicobacter 0119
Bacteroides 0085
Muribaculaceae_ge 0002
Akkermansia 0029
Lachnospiraceae_UCG-001 0027
Clostridiales_vadinBB60_group_ge 0025
Lachnospiraceae_FCS020_group_ge 0071
Mollicutes_RF39_ge 0015
Lachnospiraceae_NK4A136_group 0004
Oscillibacter 0017
Acetatifactor 0020
Ruminococcaceae_unclassified 0009
Ruminiclostridium_9 0014
GCA-900066575 0016
Ruminiclostridium_5 0019
Lachnospiraceae_unclassified 0001
uncultured 0006
A2 0010
Ruminiclostridium 0022
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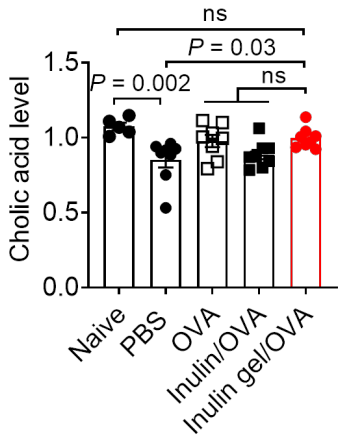
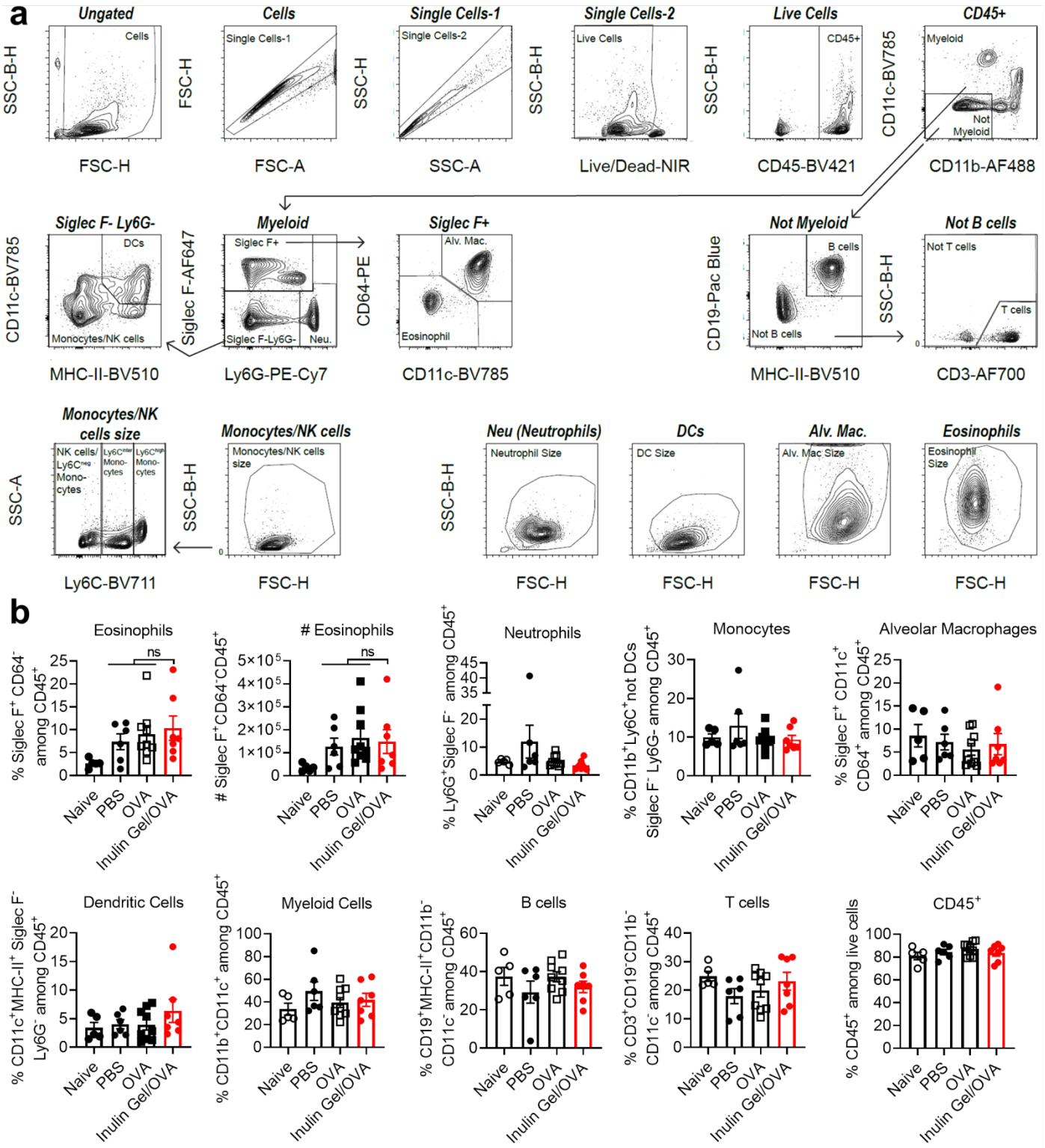
Pelomonas 0210
Staphylococcus 0060
Terrisporobacter 0033
Clostridium_sensu_stricto_1 0026
Romboutsia 0118
Streptococcus 0021
N-Acetylneuraminate
Guanosine
Homocysteic acid
L-Homoserine
Thymine
Vanilmandelic acid
5,6-dihydrouracil
1-Methylhistamine
2-Oxoisoleucine
Tetradecanedioic acid
Urea
S-Ribosyl-L-homocysteine
Choline
Trehalose-6-Phosphate
4-hydroxyphenylpyruvic acid
Mannose
Glucose
Inositol
N-Isovalerylglycine
Phenylglyoxylic acid
L-Tartaric acid
Glycerate
Cysteine-S-sulfate
N-Acetyl-aspartate
Creatine
Oxypurinol
Dopamine
p-Aminobenzoic acid
Methylnicotinamide
OMP
Biotin
Pseudouridine
Fumarate
Threonine
3-Hydroxyisovaleric acid
Propionate
Shikimic acid
2,3-Dihydroxybenzoic acid
Homogentisate
MOPEG Sulfate
2-Oxobutyrate
2-Ketohexanoic acid
Isovaleric acid
Caffeine
Quinolinic acid
2-Aminobenzoic acid
2-Hydroxyglutarate
Norepinephrine
L-Lysine
D-Sedoheptulose 7-phosphate
N-acetylornithine
L-Arginine
Homoserine
Xanthurenic acid
2,3-Butanediol
Pantothenic acid
Leucine
Cytosine
Isoleucine
Purine
Betaine
3-Methylamino-L-alanine
Malic acid
2-Hydroxyisobutyrate
4-Pyridoxate
2,3-Diphospho-D-glyceric acid
Salicylurate
Allantoin
Malondialdehyde
Glycolate
Xylonate
Ornithine
Theobromine
Methionine sulfoxide
Carbamoyl phosphate
Serotonin
Phosphoenolpyruvate
Adenine
Pyridoxamine
Taurine
Erythrose-4-phosphate
N-Acetylmethionine
2-Deoxyglucose-6-phosphate
Arabitol
Glucoronate
L-2-aminobutyric acid
Dimethylglycine
Urate
Fructose
Galactose
Myoinositol
Threonic acid
Xylose
Hydrocinnamic acid
Erythronic acid
AICAR
Indole-3-acetic acid
L-Alanine
Sarcosine
Xanthine
Hypoxanthine
Allopurinol
5-Thymidylic acid
Indole-3-propionate
Uridine
5-methyluridine
Methylmalonate
Succinate
Picolinic acid
Betaleucine
Ascorbic acid
Gentisate
Palmitic acid
4-Hydroxybenzoic acid
Acetoacetate
Oxoglutarate
3-Methyl-2-Oxovaleric
3-Methyl-3-hydroxyglutaric acid
N-Acetylglutamine
L-Methionine
Guanine
L-Histidine
L-Glutamine
Pipecolate
Citrulline
Trimethylamine
N6-Acetyl-lysine
Indole-3-lactate
Geranyl-pyrophosphate-HPO3
Phenylacetylglutamine
Deoxyguanosine
2-Hydroxybutyrate
5-Aminolevulinic acid
UTP
3-Methylphenylacetic acid
CMP
N-Acetylglycine
Phenylpyruvate
Isocitric acid
Imidazoleacetic acid
Creatinine
4,5-Dihydroorotate
Hydroxyproline
Gluconate
Cystathionine
N-Acetyl-glucosamine

Supplementary Fig. 13. Alum/OVA-sensitized BALB/c mice were treated as in **Fig. 1b**. The ileal contents were collected on day 49 for microbiome and metabolomics analyses. Shown is association study between ileal metabolomics and microbial data at genus level via machine learning. Only features that show up in any of the significant clusters are shown on the heatmap (Hallagram). Block associations are numbered in descending order of statistical significance.



Supplementary Fig. 14. Alum/OVA-sensitized BALB/c mice were treated as in **Fig. 1b**. Shown is the cholic acid level in the feces on day 48. Inulin gel (55 mg)/OVA OIT did not cause any significant changes in the levels of cholic acid in feces. Data represent mean ± s.e.m. (n = 5 for Naive, 8 for all other groups biologically independent samples). ns: not significant. Data were analysed by one-way ANOVA with Bonferroni's multiple comparisons test.



Supplementary Fig. 15. Mice were treated as shown in **Fig. 1b**. The lungs were isolated on day 61 and analyzed: **a**. Gating strategies for various immune cells; **b**. The frequencies of various immune cells, including eosinophils (Siglec $F^+CD64^-CD45^+$ cells). Data represent mean ± s.e.m. (n = 5 for Naive, 6 for PBS, 9 for OVA, or 7 for Inulin gel/OVA biologically independent samples). ns: not significant. Data were analysed by one-way ANOVA with Bonferroni's multiple comparisons test.

Product #	D12450J		D15092907	
	gm%	kcal%	gm%	kcal%
Protein	19	20	16	20
Carbohydrate	67	70	47	58
Fat	4	10	4	10
kcal/gm	3.8		3.2	
Ingredient	**gm**	**kcal**	**gm**	**kcal**
Casein	200	800	200	800
L-Cystine	3	12	3	12
Corn Starch	506.2	2025	383.25	1533
Maltodextrin 10	125	500	125	500
Sucrose	68.8	275	68.8	275
Cellulose, BW200	50	0	50	0
Inulin	0	0	328	492
Soybean Oil	25	225	25	225
Lard	20	180	20	180
Mineral Mix, S10026	10	0	10	0
DiCalcium Phosphate	13	0	13	0
Calcium Carbonate	5.5	0	5.5	0
Potassium Citrate, 1 H_2O	16.5	0	16.5	0
Vitamin Mix, V10001	10	40	10	40
Choline Bitartrate	2	0	2	0
FD&C Yellow Dye #5	0.05	0	0	0
FD&C Red Dye #40	0	0	0.05	0
Total	**1055.05**	**4057**	**1260.1**	**4057**
Total Fiber (%)			30.00	

Supplementary Table 1. Composition of high inulin chow diet (D15092907) and control chow diet (D12450J).

References for Supporting Information

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