Supplementary Materials

Supplementary methods

Animal experiments

For co-housing experiments, age-matched female mice were either left untreated or treated with CS inhalation two times daily, and kept in: isolated cages [control (CTL) and CS); or cohoused [CO-CS (CTL cohoused with CS) and CO-CTL (CS cohoused with CTL)] at a 1:1 ratio for 12 weeks. CS mice were put into a smoking chamber and treated with smoking, while CTL group was only put into chambers without smoking.

For antibiotics treatment, a modified protocol from Wu et al.[1] was used. Mice were either orally treated with PBS (CTL), antibiotic cocktail (ABX: 0.5 g/l ampicillin, 0.5 g/l metronidazole, 0.5 g/l neomycin sulfate and 0.25 g/l vancomycin; Sigma, St. Louis, USA), VAN, AMP, NEO, or MET at the same concentration in sterile drinking water. Antibiotics treatments were performed for two weeks before CS inhalation and continued for the entire experimental duration.

For FMT experiments, donor mice (8-week old, female) were either untreated, or treated with CS inhalation, and with/without ABX/ single antibiotic by drinking water for 12 weeks. Between week 10 -12 (15 days), fecal matter of each mice was collected daily (150–180 mg), pooled, stored in sterile tubes and homogenized in 1 ml of PBS. After centrifugation (2,000g at 4°C for 1 min), bacteria-enriched supernatants were collected and centrifuged (5 min at 15,000g). Bacterial pellets were washed twice with PBS, resuspended in 700 µl of saline with 20% (v/v) glycerol, and stored at –80°C. CS mice were treated daily with fecal microbiota transplants (ca. 1x 10^8 CFU in 100 µl PBS) from each donor group (8-week old, female) via oral gavage for 12 weeks.

For *P. goldsteinii* MTS01 experiments, mice were divided into three groups. The control group with PBS oral gavage once per day, comprised mice exposed to room air for

twelve weeks. Two CS groups were exposed to smoke for twelve weeks. Each group was once per day, either orally gavaged with $2x10^8$ CFU *P. goldsteinii* MTS01 or PBS, respectively.

For evaluation of LPS ameliorative effect, CTL or CS mice were intraperitoneal (*i.p.*). injected with 100 µl PBS or high (100 µg/kg)/ low (10 µg/kg) dose of LPS isolated from *P. goldsteinii* MTS01 (Pg-LPS). At prior-indicated timepoints, mice were euthanized and sacrificed for characterization.

Bronchoalveolar lavage

BAL fluid (BALF) was obtained using a 20 G intravenous catheter inserted into the trachea (with 1 ml PBS). The fluid was centrifuged (1,000g for 3 min) and pellets were resuspended in 1 ml PBS. Total and differential cell-counts were determined using a hemocytometer and a Diff-Quick staining kit (Sysmex, Kobe, Japan).

Flow cytometry analysis

We followed Wu et al.'s protocol [1] using the following antibodies purchased from BD Pharmingen (USA): CD11c-PE (HL3), F4/80-PE (BM9), Ly6G-PE (1A8), Ly6C-FITC (AL-21), CD19-PE (1D3), CD4-PE (GK1.5), γδ TCR-FITC (GL3), TNF-α-APC (MP6-XT22), FoxP3-FITC (FJK-16s), and IL-17A-Alexa Fluor647 (TC11-18H10); IL-10-APC (JES5-16E3) from eBioscience (San Diego, USA); IL-1β-APC from R&D Systems (Minneapolis, USA); and Rabbit IgG-FITC from Beckman Coulter (Brea, USA). For cell surface staining, single cell suspensions were stained with antibodies (1:100), washed once with cold PBS containing 2% FBS and resuspended in cold PBS. For surface and intracellular staining, cells were incubated in RPMI-1640 medium containing phorbol 12-myristate 13-acetate (PMA, 25 ng/ml, Sigma) and ionomycin (1 µg/ml, Sigma) for 1 h, followed by GolgiPlug (BD Pharmingen) addition and 3 h-

incubation at 37°C under 5% CO_2. Stimulated cells were surface antibody-stained (1:100) and fixed and permeabilized using the Cytofix/Cytoperm kit (BD Pharmingen) following manufacturer's instructions, followed by intracellular staining (1:50). All stained cells were analyzed using FACS Calibur (BD, USA). Data were analyzed using Kaluza (v1.5, Beckman Coulter, USA) or FlowJo (v10.4, USA).

Histological analysis

Lungs were formalin-fixed and paraffin-embedded. Tissue sections were stained with hematoxylin and eosin (H&E) and examined under a light microscope (Olympus, Tokyo, Japan). Histological images were analyzed using the Image J software (National Institutes of Health, Bethesda, USA). Two randomly-selected fields from each 10–15 sections were analyzed.

Assessment of pulmonary functions

We used Vanoirbeek et al.[2] protocols, involving mice whole-body plethysmography, anesthetization, tracheostomization, and placement in a forced pulmonary maneuver system (Buxco Research Systems). The anesthetized animals were induced to have 100 breaths/min on average. Quasistatic PV maneuvering was performed to measure chord compliance (Cchord).

Microbiota library construction and Illumina MiSeq sequencing

Feces samples were snap-frozen in liquid nitrogen and stored at –80°C. DNA was extracted using a QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany). BALF samples were centrifuged at 5,000g for 10 minutes to collect the pellet and DNA was extracted using a QIAamp DNA mini kit (Qiagen, Hilden, Germany). V3-V4 regions in the 16S rRNA gene were amplified by using primers listed in supplementary table

S1, purified and sequenced using the MiSeq Illumina pyrosequencer platform following manufacturer's instructions.

16S rDNA-based metagenomics analysis pipeline

We used a protocol modified from our previous studies [1, 3, 4] where V3-V4 regions of 16S rDNA amplicon (forward primer 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG*CCTAYGGGRBGCASCAG*-3'; reverse primer 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG*GGACTACNNGGGTATCTAAT*-3') sequencing was performed using 250 bp paired-end raw reads and the entire paired-end reads were assembled using FLASH v.1.2.11. Low-quality reads (Q score <20) were discarded in QIIME 1.9.1 pipeline. If three consecutive bases were <Q20, the read was truncated, and the resulting read was retained for data at least 75% of the original length (QIIME script split_libraries_fastq.py). Sequences were chimera-checked using UCHIME and filtered from the data set before OTU (operational taxonomy units) picking of 97% sequence identity using USEARCH v.7 pipeline (UPARSE function). For each representative sequence, the Silva Database v128 was used based on RDP classifier (v.2.2) algorithm to annotate taxonomy classification, which was performed with an 80% minimum confidence threshold to record an assignment. Any singleton-sequences were filtered out.

Sample-species complexity was evaluated by Beta diversity analysis. Supervised partial-least-squares discriminant analysis (PLS-DA)—R package mixOmics—was used to evaluate and visualize variance based on OTUs level of gut microbiota composition among the groups.

Statistical significance analysis for all species among various taxonomic levels was determined by differential abundance analysis with a zero-inflated Gussian (ZIG) log-

normal model as implemented in the Bioconductor metagenomeSeq package's "fitFeatureModel" function [5]. Only bacterial family with relative abundance ≥0.1% and bacterial species with relative abundance ≥0.01% in at least two samples were retained. Statistically significant biomarkers were identified using the LEfSe (Linear discriminant analysis (LDA) Effect Size) analysis-an algorithm-based approach that performs the non-parametric Kruskal-Wallis test and Wilcoxon rank-sum test to identify bacterial taxa whose relative abundance is significantly different relative to the control [6]. LEfSe applies LDA to bacterial taxa identified as significantly different and assesses the effect size of each differentially abundant taxon. Taxa with LDA score (log10) > 4 was considered significant.

P. goldsteinii cultivation

P. goldsteinii MTS01 was isolated from feces of mice in ABX experiments. Bacteria were grown at 37 °C in a Whitley DG250 anaerobic chamber (Don Whitley, Bingley, UK) with mixed anaerobic gas (5% carbon dioxide, 5% hydrogen, 90% nitrogen). Anaerobic condition was confirmed using an anaerobic indicator (Oxoid, Hampshire, UK). *P. goldsteinii* MTS01 was cultivated on anaerobic blood agar (Creative, New Taipei city, Taiwan) and liquid thioglycollate medium (BD, Franklin Lakes, USA).

Whole genome sequencing and analysis of _P. goldsteinii_ MTS01

Illumina and Pacbio platforms were used for *P. goldsteinii* MTS01's full-genome sequencing by hybrid assembly. The main steps were as follows: Pacbio platform reads were first assembled by Canu [7]. The contigs were mapped by Pacbio reads using minimap2 [8] and corrected, then assembled by Racon [9]. Short reads were then mapped to the assembled contigs by BWA [10], and Pilon[11] was applied for sequence correction. Open reading frame was predicted by MetaGeneMark [12]. Predicted genes

were annotated by: KEGG [13], COG [14], NCBI-NR. Blast [15] search was performed against KEGG and NCBI-NR databases. COG database search was done by CD-search [16]. All software were processed in default.

Sera metabolomics analysis

The sera metabolites were analyzed by GC-MS and LC-MS. An Agilent 7890 gas chromatograph system coupled with a LECO Pegasus BT time-of-flight mass spectrometer was used for analysis. MS-DIALsoftware [17] and FiehnBinbase databases were used raw peak-extraction, baseline data filtration and calibration, peak alignment, deconvolution analysis, peak-identification and peak-area integration [18]. For metabolites identification, both mass spectra and retention index matches were considered. We removed peaks detected in ≤50% of QC samples or <50% samples of every group except QC group or RSD＞30% in QC samples [19]. Meanwhile, extracted sera samples were analyzed by an UHPLC system (1290, Agilent Technologies) with a UPLC BEH Amide column (1.7μm 2.1*100mm, Waters) coupled to TripleTOF 6600 (Q-TOF, AB Sciex). Peak annotation was done by R package CAMERA post-XCMS data processing an in-house MS2 database was applied for metabolites identification. Supervised Orthogonal Projections to Latent Structures Discriminate Analysis (OPLS-DA) utilized three-dimensional data consisting peak number, sample name, and normalized peak area. Statistical-multivariate analyses (OLPS-DA) were performed by R package ropls to distinguish test-subjects and controls. The OPLS-DA model was applied for higher-level group separation and comprehension of variables responsible for classification. The model's predictive ability was assessed by a 7-fold cross-validation method. The classification parameters, R2Y (the goodness-of-fit) and Q2 (goodness-of-prediction) intercept values (200 permutations) were 0.918 and 0.745 (GC), 0.980 and 0.627 (LC-positive ion mode), 0.566 and 0.792 (LC-negative ion

mode), respectively, indicating the model's robustness, a low over-fitting risk, and good reliability. For analysis refinement by first principal component of variable importance projection (VIP), the significantly different metabolites were only acquired on VIP > 1 and Student's t-test p-value < 0.05 among the pairwise comparison groups. The significant metabolites were used to analyze canonical pathway enrichment analysis using Ingenuity Pathway Analysis (IPA, QIAGEN bioinformatics, Germany).

scRNAseq analysis of colon and lung tissues

The raw scRNA-Seq data were de-multiplexed, quantified and grouped by 10x Genomics' Cell Ranger Single-Cell Software Suite. The quantified results were normalized by Seurat v3 [20] and individual cell types determined using SingleR[21]. The differentially expressed genes (adjusted p-value<0.05, average log(FoldChange)>0.4 >0.4 or <-0.4) were identified with (http://comphealth.ucsf.edu/SingleR), relying on default Seurat's DEGs identification methods. Mouse annotation information was obtained from the org.Mm.eg.db package [22]. ClusterProfiler, ComplexHeatmap, and ggplot2 packages were used for pathway enrichment analysis, sub-setting cell types and heatmap-generation or pathway-enrichment plots [23, 24]. We presented 15 most significant *GO* terms (adjusted p-value<0.05).

Cellular bioenergetic analysis

Bioenergetic analysis was performed on intact cells using the XF24 analyzer (Seahorse Bioscience, Billerica, MA, USA). Real-time measurements for oxygen consumption rate (OCR)-mitochondrial-respiration indicator, and extracellular acidification rate (ECAR)-a glycolysis index, were taken on colon tissues from mice subjected to various treatments.

Genes expression levels

Lung and colon tissues were harvested from the mice and total RNAs extracted using the Genezol TriRNA pure kit (Geneaid, New Taipei City, Taiwan). RNA was reversely-transcribed using the Quant II fast reverse transcriptase kit (BioTools, New Taipei city, Taiwan). The resulting cDNA (1 µl) was used as template for qPCR and mixed with 1 µl of target gene primers (supplementary table S1), 5 µl of 2x qPCRBIO SyGreen Blue Mix Lo-ROX (PCR Biosystems, London, UK) and 3 µl double distilled water in each well. The GAPDH primers were used as the internal control for qPCR assay. The PCR conditions were as follows: initial pre-incubation-step at 95 °C for 3 min, 50 PCR cycles at 95 °C for 10 sec, 60 °C for 20 s, 72 °C for 5 s and one melting curve cycle.

Lung immunoglobulin measurement

Supernatants were collected from centrifuged (15,000g for 1 min) lung-tissue homogenates and IgG was quantified by ELISA kits following manufacturer's instructions (R&D Systems).

Endotoxin detection

BALF and serum LPS were measured using a murine HEK-Blue™ LPS Detection *Kit* (InvivoGen, USA) based on the manufacturer's instructions.

***P. goldsteinii* LPS purification**

LPS were isolated by using the hot phenol-water extraction [25]. Bacterial pellet of 1200 ml overnight culture were suspended in 30 ml of the warm water and added an equal volume of phenol, then stirred at 65°C for 30 minutes. After centrifugation, the aqueous layers were collected, and the organic layer were added an equal volume of °

warm water to perform the extraction twice. The crude extract obtained after dialysis and lyophilization were treated with DNase, RNase and Proteinase K. Further dialysis and lyophilization were performed to obtain the purified LPS.

Antagonistic effect in HEK-Blue-mTLR4 cells

HEK-Blue-mTLR4 cells (InvivoGen, USA) were pretreated with Pg-LPS for 2 hours, followed by 20 h-EC-LPS (*E. coli* O111:B4 LPS purchased from Sigma) treatment. The NF-kB activation was determined by measurement of OD630. The binding competition of 0.6 μg/ml FITC-EC-LPS (FITC-*E. coli* O111:B4 LPS purchased from Sigma) on HEK-Blue-mTLR4 cells with pretreatment of different amounts of Pg-LPS was analyzed by flow cytometry.

Cell isolation and *in vitro* LPS treatment

CD11c+ DC were isolated from GM-CSF-stimulated murine bone marrow cells using the EasySep^{TM} mouse CD11c positive selection kit following supplier's instructions (Stemcell Technologies, Vancouver, Canada). Human PBMC were isolated by SepMate PBMC isolation tube per instructions (Stemcell Technologies, Vancouver, Canada). Cells were maintained in RPMI Medium (Gibco, Waltham, USA) supplemented with 10% FBS, and 1% antibiotic-antimycotic (Thermo Fisher Scientific, USA) at 37°C under 5% CO_2. For LPS treatment, cells were seeded (CD11c+ DC: 2×10^5 cells, human PBMC: 1×10^6 cells) and pretreated with Pg-LPS for 2 hours, followed by 24 h-EC-LPS (*E. coli* ultrapure O111:B4 LPS purchased from InvivoGen) treatment. TNF-α or IL-1β were detected by ELISA from supernatants.

B-cell isolation

B cells were isolated from spleen single cells using the EasySep^{TM} mouse B-cell

isolation kit following supplier's instructions (Stemcell Technologies, Vancouver, Canada). Flow-cytometry (FACSAria, BD) confirmed isolation recovery rate and B-cell purity. B cells were resuspended (4×10^7/ml) in flow cytometry staining buffer (PBS containing 2% FBS) and stained with a final concentration of 5 μM 5-(and-6)-carboxyfluorescein diacetate succinimidyl ester (CFDA-SE)(Molecular Probes, Eugene, USA) at 37°C for 10 min. Following which cells were immediately washed three-times with cold flow cytometry staining buffer. Resuspended cells (in culture medium) were seeded in a 24-well plate (2×10^6 cell/1 ml). LPS-stimulated cell proliferation was examined by flow cytometry after 48 hr.

Database deposition

The 16S sequence reads of antibiotics- and FMT-treated mice groups have been deposited under NCBI BioProject numbers PRJNA679392 and PRJNA680828, respectively. The single cell sequence reads have been deposited under the NCBI BioProject number PRJNA680859. The genome sequences and annotation of *P. goldsteinii* MTS01 strain have been deposited at NCBI under the BioProject number PRJNA674720.

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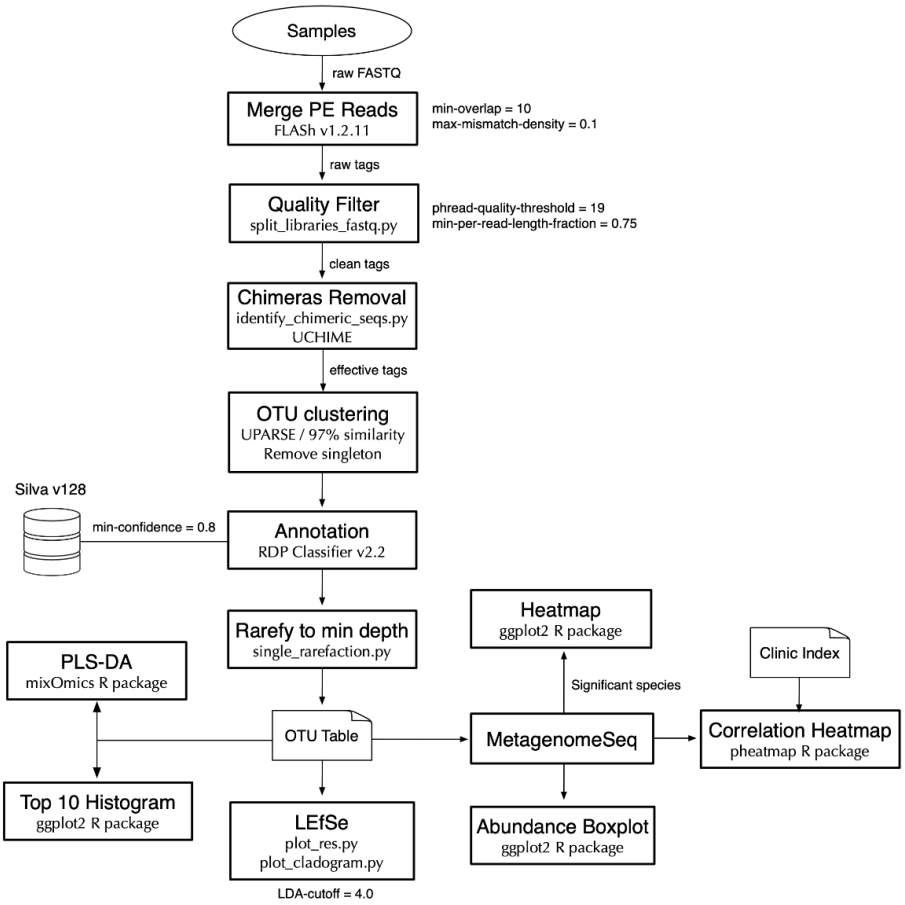
Supplementary Table S1. Primers used in this study**.**

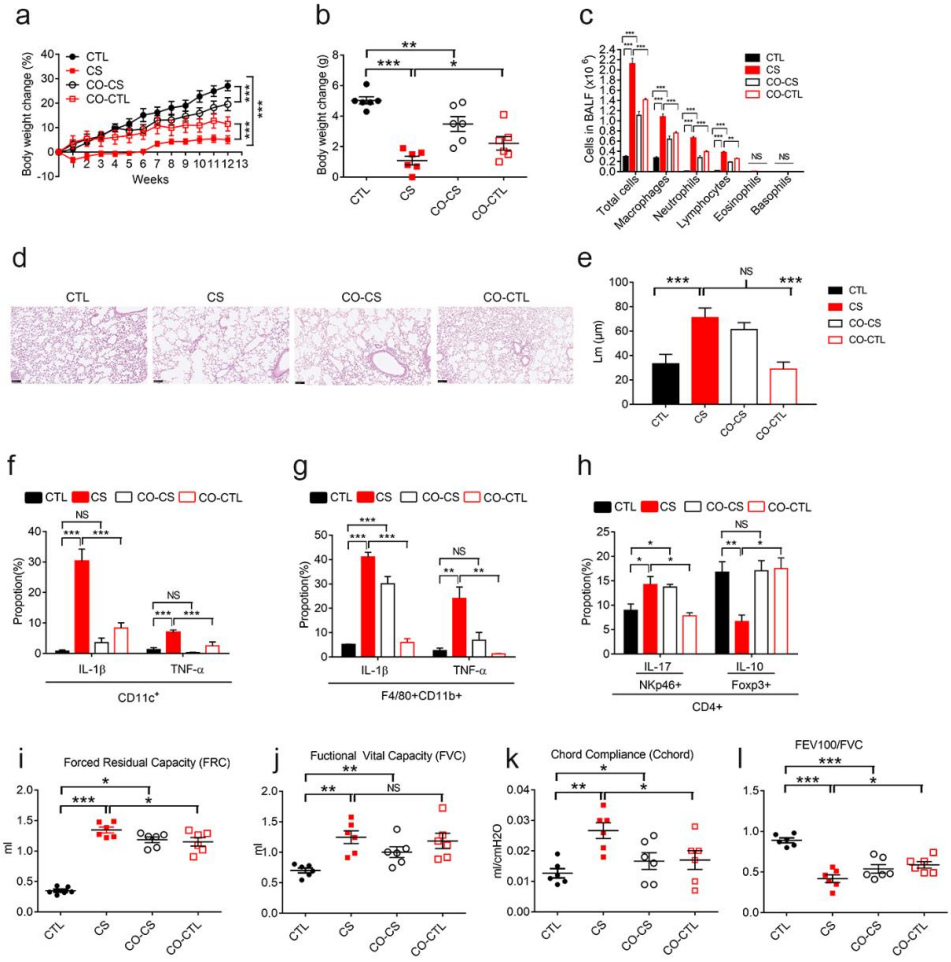
Target		Primer sequence (5'–3')	Slope	Efficiency (%)	Y-intercept
IL-1β	Forward	TTGAAGAAGAGCCCATCCTC	-3.604	89.44	37.46
	Reverse	CAGCTCATATGGGTCCGAC			
TNF-α	Forward	TAGCCAGGAGGGAGAACAGA	-3.688	86.70	39.05
	Reverse	TTTTCTGGAGGGAGATGTGG			
ZO-1	Forward	ACCCGAAACTGATGCTGTGGATAG	-3.717	85.80	39.24
	Reverse	AAATGGCCGGGCAGAACTTGTGTA			
Occludin	Forward	ATGTCCGGCCGATGCTCTC	-3.178	106.38	35.68
	Reverse	TTTGGCTGCTCTTGGGTCTGTAT			
Mptx1	Forward	CTCTGTTCTTTCAGGAAGTGTAGC	-3.822	82.66	41.16
	Reverse	CAGTGACTTCCTCACCTTCGGT			
Ang4	Forward	GGCACCAAGAAAAACATCAGGGC	-3.423	95.95	36.56
	Reverse	GTGCGTACAAGTGGTGATCTGG			
Muc2	Forward	CCCAGAAGGGACTGTGTATG	-3.589	89.95	37.62
	Reverse	TTGTGTTCGCTCTTGGTCAG			
RNR1	Forward	ACCGCGGTCATACGATTAAC	-3.325	99.87	36.51
	Reverse	CCCAGTTTGGGTCTTAGCTG			
RNR2	Forward	CCGCAAGGGAAAGATGAAAGAC	-3.393	97.12	36.88
	Reverse	TCGTTTGGTTTCGGGGTTTC			
Cytb	Forward	ATTCCTTGATGTCGGACGAG	-3.106	109.87	35.41
	Reverse	ACTGAGAAGCCCCCTGAAA			
PGC-1α	Forward	TATGGAGTGACATAGAGTGTGCT	-3.510	92.71	38.08
	Reverse	CCACTTCAATCCACCCAGAAAG			
ND-5	Forward	AGCATTCGGAAGCATCTTG	-3.333	99.54	36.43
	Reverse	TTGTGAGGACTGGAATGCTG			
TFAM	Forward	CACCCAGATGCAAAACTTTCAG	-3.433	95.56	36.89
	Reverse	CTGCTCTTTATACTTGCTCACAG			
Scgb1a1	Forward	GGTTATGTGGCATCCCTGAAGC	-3.306	100.67	36.46
	Reverse	GCTTACACAGAGGACTTGTTAGG			
GAPDH	Forward	GCATCCACTGGTGCTGCC	-3.372	97.95	38.63
	Reverse	TCATCATACTTGGCAGGTTTC			
16S V3/V4	Forward	CCTACGGGNGGCWGCAG	-3.757	84.57	40.30
	Reverse	GACTACHVGGGTATCTAATCC			

PG 16S	Forward	GAATAAAGTGAGGAACGTGTT	-3.621	88.87	38.66
	Reverse	AACTTTCACCGCTGACTTAATTA			
EC 16S	Forward	GGAAGAAGCTTGCTTCTTTGCTGAC	-3.741	85.06	38.44
	Reverse	AGCCCGGGGATTTCACATCTGACTTA			

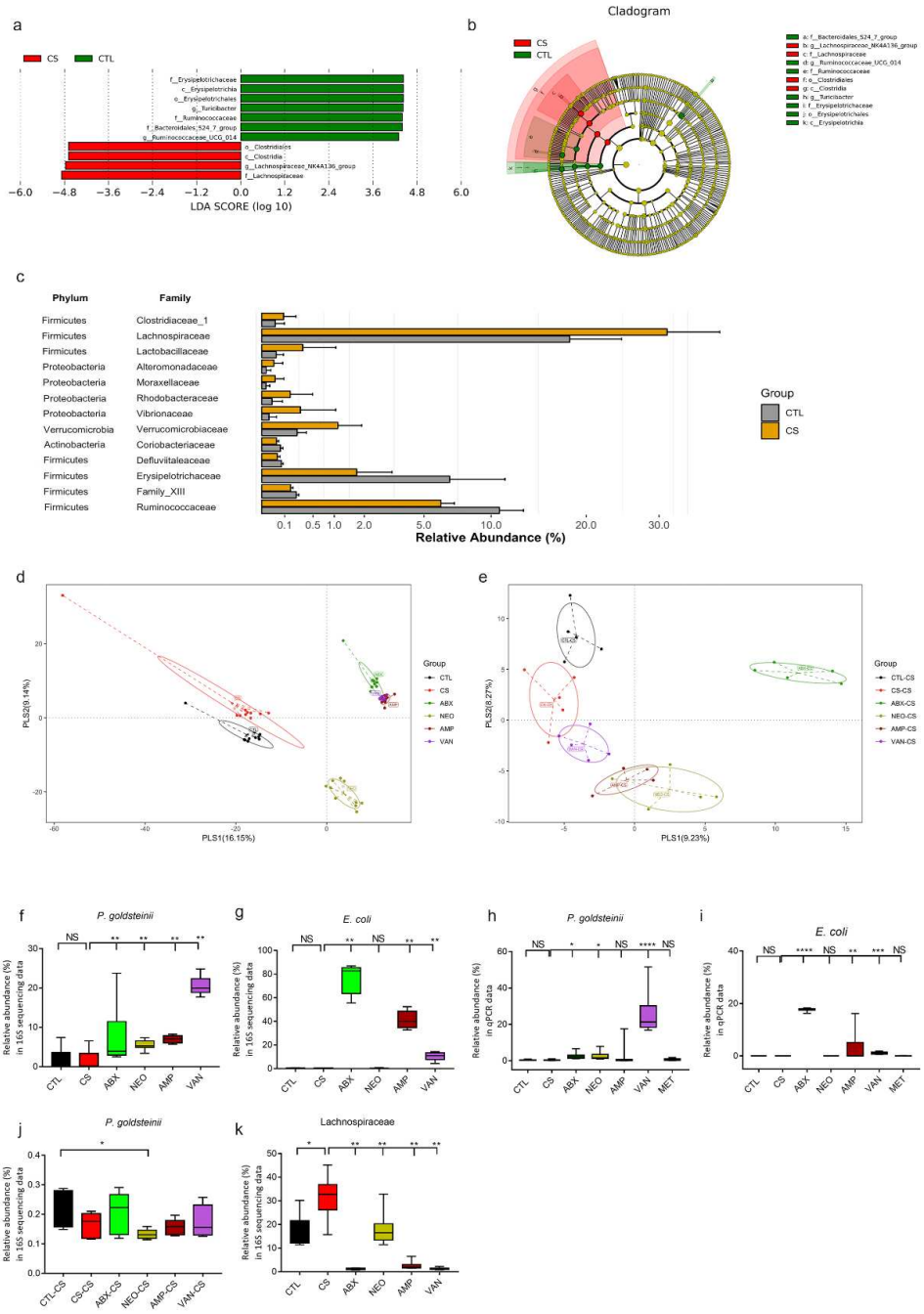
*Dimer formations of primers were predicted by using OligoEvaluator provided by Sigma-Aldrich (http://www.oligoevaluator.com/LoginServlet). The prediction revealed that no primer dimers were formed.



Supplementary Figure S1. The workflow of 16S rDNA-based metagenomics analysis pipeline. The detailed methods can be found in supplementary methods.

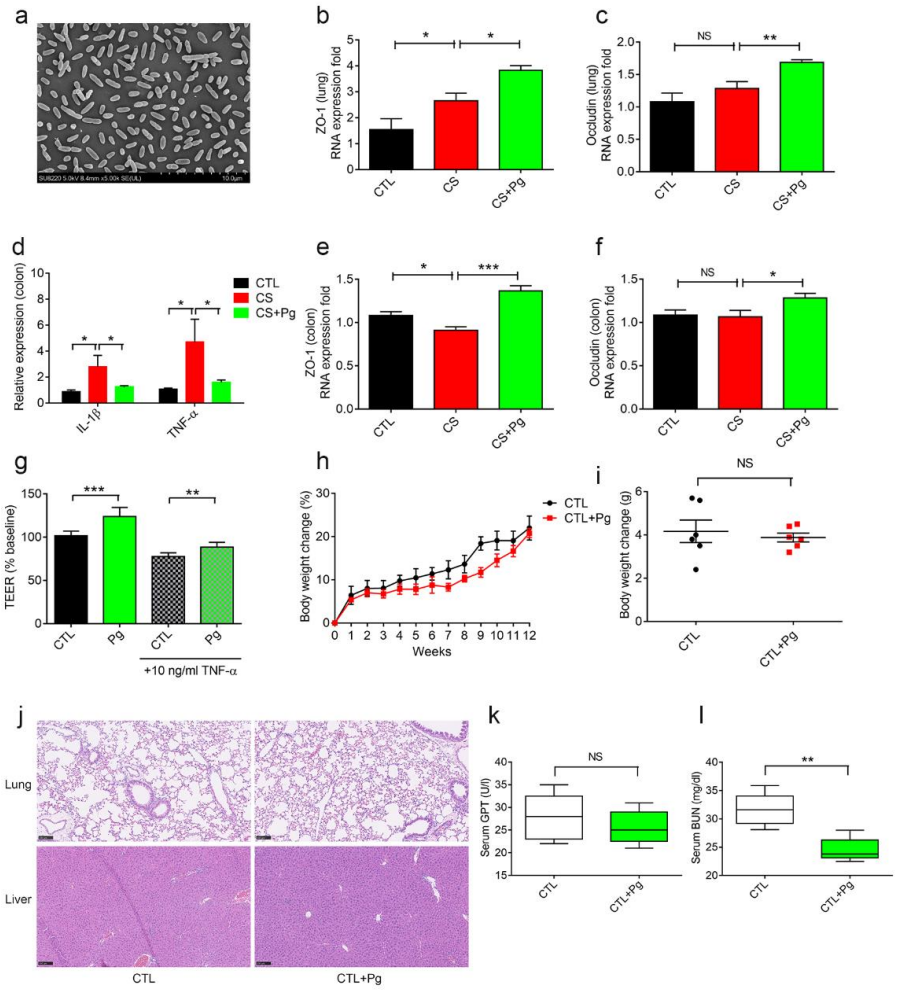


Supplementary Figure S2. Effects of co-housing on development of COPD. To evaluate the effect of cohousing treatment on COPD development in CS mice, control (CTL) and CS mice were cohoused with CS (CO-CS) and control (CO-CTL) mice, respectively, following the protocols described. Percentage of body weight change was measured throughout the 12-week period (a). Body weight change (b), BALF cellular composition (c), histopathology in lung (d), the mean linear intercept of lung tissue (e), flow cytometry analysis on production of IL-1β and TNF-α in CD11c+ cells (f), F4/80+CD11b+ macrophages (g), IL-17A in CD4+NKp46+ Th17 cells, and IL-10 in CD4+FoxP3+ Treg cells (h), and lung functions analyses including FRC (i), FVC (j), Cchord (k), and FEV100/FVC (l) were measured after 12 weeks. Except panel d, statistical analysis was performed using the one-way ANOVA followed by non-parametric Kruskal-Wallis test with Newman-Keuls multiple comparison (n=6). *, P<0.05; **, P<0.01; ***, P<0.001. NS, not significant.

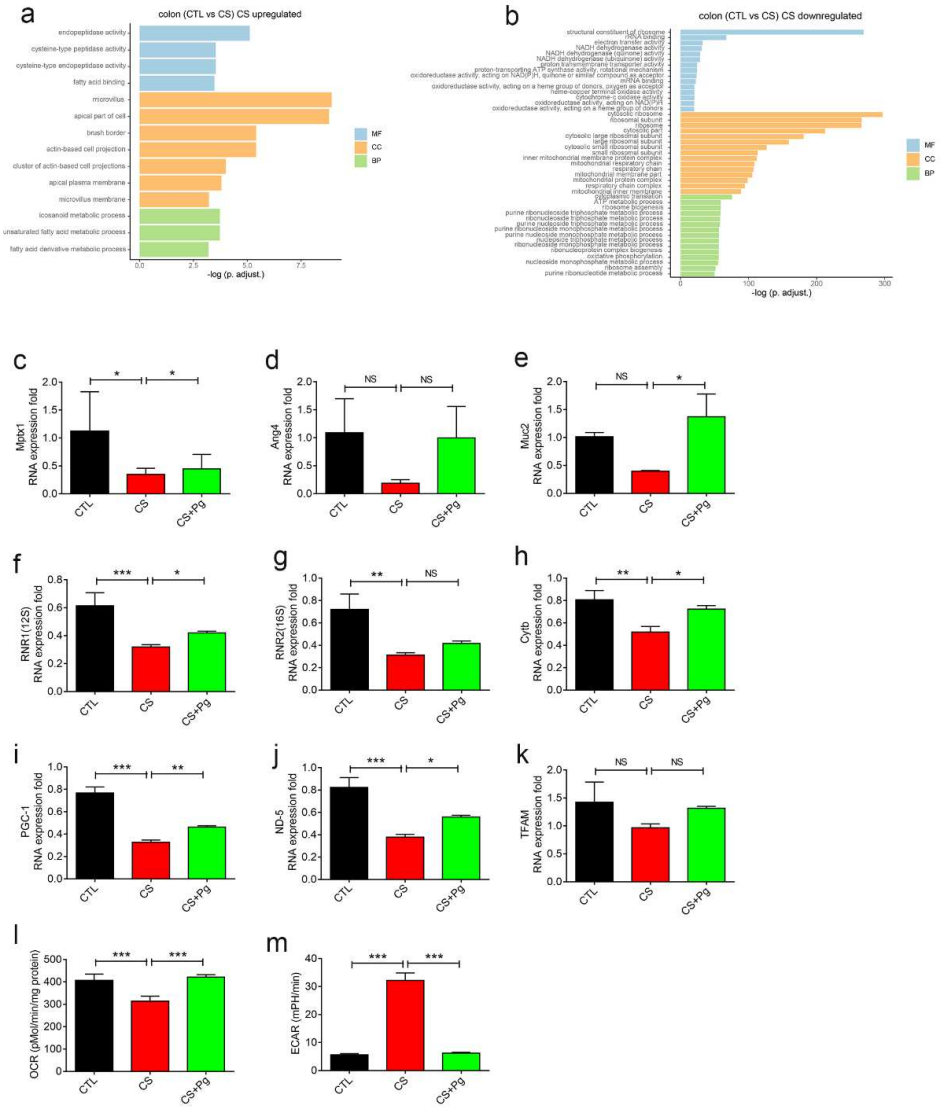


Supplementary Figure S3. *In silico* analysis of faeces microbiota from different mice groups. Faeces of different mice groups were collected, followed by DNA extraction, sequencing and bioinformatics analysis. The most significant differentially abundant taxa between CTL and CS groups were identified by LEfSe analysis (a). CTL-enriched taxa were indicated with a positive logarithmic LDA score (green), and taxa enriched in CS group with a negative score (red). Only taxa meeting the criteria for

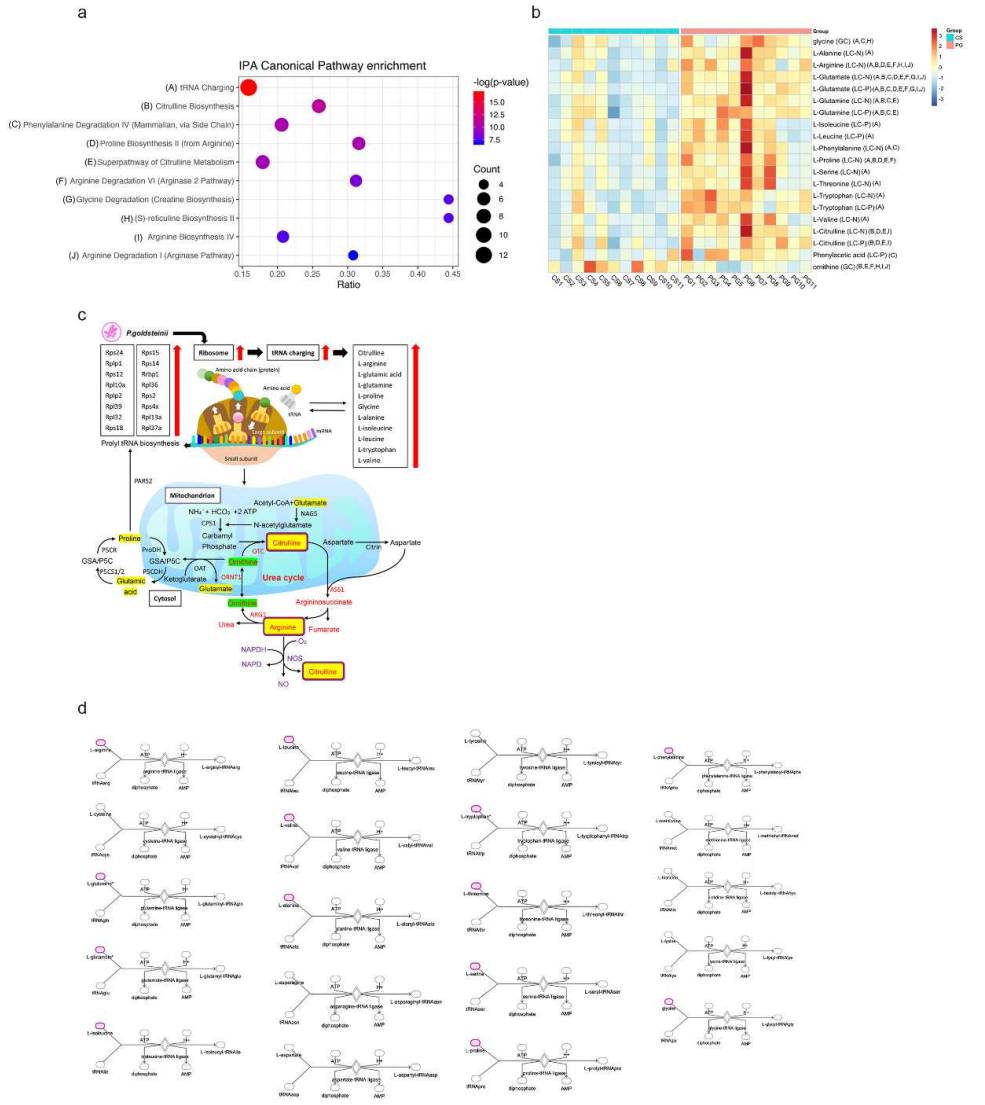
feature selection in LDA score (log10) > 4.0 with P < 0.05 were shown. A taxonomic cladogram representation of data was shown in panel (b). Red, CS-enriched taxa; green, taxa enriched in CTL group. The size of each dot is proportional to its relative abundance. The relative abundance of significantly different bacterial families between CTL and CS mice (q<0.05) analyzed by metagenomeSeq was also shown in (c). (d) The β-diversity (expressed as PLS1 and PLS2) of microbiota bacteria taxa in CTL, CS, ABX, NEO, AMP and VAN groups analyzed by PLS-DA was shown. (NEO, neomycin, AMP, ampicillin, VAN, vancomycin, MET, metronidazole, ABX, combination of the four antibiotics.) (e) After FMT, the β-diversity (expressed as PLS1 and PLS2) of microbiota bacteria in recipient groups of CTL-CS, CS-CS, ABX-CS, NEO-CS, AMP-CS and VAN-CS mice analyzed by PLS-DA was shown. Relative abundance of *P. goldsteinii* (f, h) and *E. coli* (g, i) in CTL, CS, antibiotics-treated groups of 16S sequencing data (**, q<0.01; NS, not significant) and qPCR validation (*, P<0.05; **, P<0.01; ***, P<0.001; ****, P<0.0001. NS, not significant) was shown. The relative abundance of *P. goldsteinii* (j) in FMT-treated groups of 16S sequencing data was shown (*, P<0.05). Relative abundance of Lachnospiraceae family (k) in CTL, CS, antibiotics-treated groups of 16S sequencing data was shown (*, q<0.05; **, q<0.01).



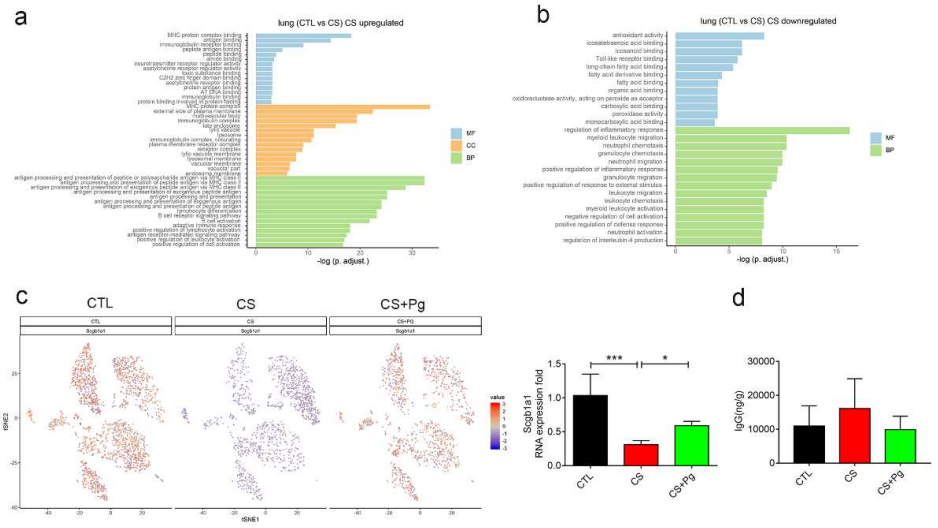
Supplementary Figure S4. Electron micrograph (EM) of *P. goldsteinii* MTS01 and its effect on CS and CTL mice. The EM morphology of *P. goldsteinii* MTS01 under the scale bar of 10 μm was shown in (a). Effects of *P. goldsteinii* MTS01 administration on expression level of ZO-1 (b) and occludin (c) in lung, IL-1β and TNF-α (d) in colon and ZO-1 (e) and occludin (f) in colon of CS mice were shown. The effects of *P. goldsteinii* MTS01 on TEER of Caco-2 cells under TNF-α (10 ng/ml) treatment was shown in (g). Effects of *P. goldsteinii* MTS01 on body weight change (h, i), histopathology in lung and liver (j), serum GPT (k) and BUN (l) levels of control mice were shown. In panel b–g, statistical analysis was performed using the Newman-Keuls multiple comparison post hoc one-way ANOVA analysis (n=6-12). In panels h, i, k and l, data represent means ± standard deviation (SD) analyzed using the un-paired Student *t* test. *, P<0.05; **, P<0.01; ***, P<0.001. NS, not significant.



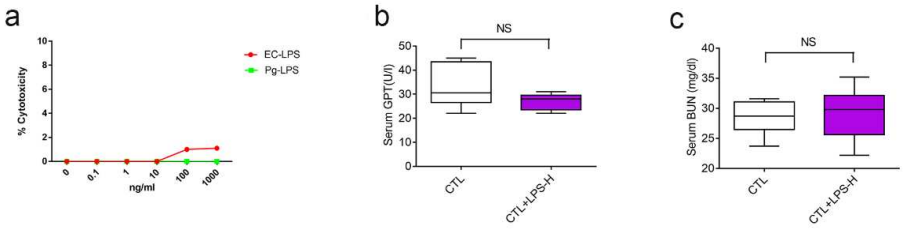
Supplementary Figure S5. Validation of results of single cell RNA sequencing (scRNAseq) analyses in colon of CTL, CS and CS mice treated with *P. goldsteinii* MTS01. Results of GO enrichment pathways derived from differentially expressed genes in colon between CTL and CS mice were shown in (a, b). The mRNA expression levels of Mptx1, Ang4, and Muc2 were shown in (c), (d) and (e), respectively. The mRNA expression levels of RNR1 (mitochondrial 12S) (f), RNR2 (mitochondrial 16S) (g), Cytb (h), PGC-1 (i), ND-5 (j) and TFAM (k) as well as oxygen consumption rate (OCR) (l) and extracellular acidification rate (ECAR) (m) measured by seahorse XF analyzers were shown. In panels c–m, statistical analysis was performed using the Newman-Keuls multiple comparison post hoc one-way ANOVA analysis (n=6). *, P<0.05; **, P<0.01; ***, P<0.001. NS, not significant.



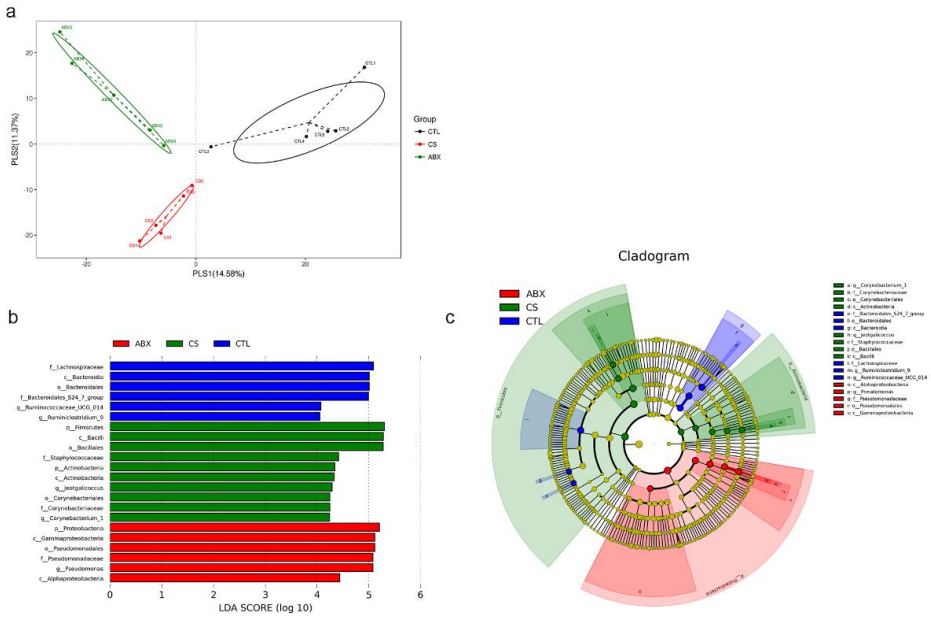
Supplementary Figure S6. Serum metabolomic analysis of CTL, CS and CS administered with *P. goldsteinii* MTS01 mice groups. The top 10 canonical enrichment pathways derived from differentially expressed sera metabolites between CS and CS+Pg group (a) were analyzed by IPA. Heatmap of differentially expressed sera metabolites between CS and CS+Pg mice groups involved in the top 10 canonical pathways enrichment were shown in (b). The cellular activities most significantly affected by *P. goldsteinii* MTS01 administration, including ribosomal, mitochondrial and urea cycle activities were shown in (c). Results of tRNA charging pathways affected by *P. goldsteinii* MTS01 administration under CS treatment (d). Those highlighted with red circles indicated amino acids significantly involved in enhanced tRNA charging activity.



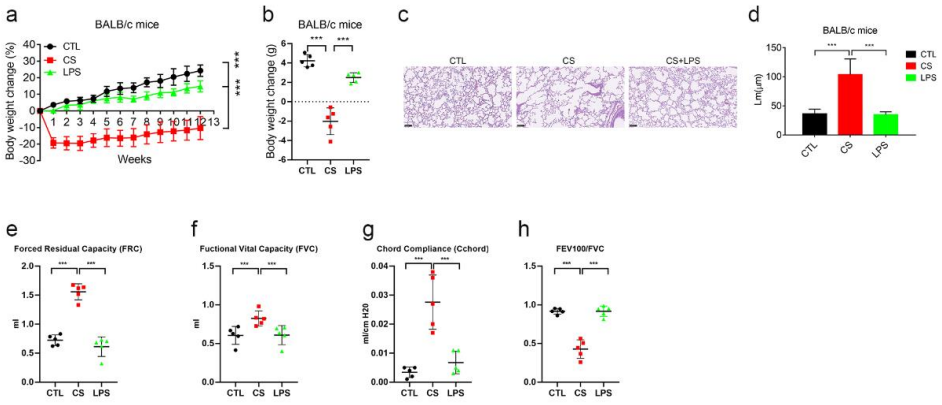
Supplementary Figure S7. Results of GO pathway enrichment analysis in lung of CTL and CS mice. Enriched pathways for upregulated and downregulated genes in CS were shown in (a) and (b), respectively. The scRNAseq results and mRNA expression level of Scgb1a1 were shown in (c). The IgG levels in lung homogenates among CTL, CS and CS+*P. goldsteinii* MTS01 were shown in (d). Quantitative of mRNA expression levels were analyzed using the Newman-Keuls multiple comparison post hoc one-way ANOVA analysis. *, P<0.05;***, P<0.001. MF, Molecular Function. CC, Cellular Component, BP, Biological Process. Pg, *P. goldsteinii* MTS01.



Supplementary Figure S8. Safety of Pg-LPS treatment *in vitro* and *in vivo*. The cytotoxicity measured by release of lactate dehydrogenase on HEK-mTLR4-blue cells after treatment of different doses of EC-LPS and Pg-LPS for 24 hours were shown in (a). Effects of Pg-LPS treatment on serum GPT (b) and BUN (c) levels in control mice were shown. In panels b and c, the data represent means ± standard deviation (SD) analyzed using the un-paired Student *t* test (n=5). NS, not significant.



Supplementary Figure S9. The composition of microbiota in BALF of CTL, CS, and ABX mice groups. The β-diversity (expressed as PLS1 and PLS2) of BALF microbiota bacteria taxa in CTL, CS, and ABX groups analyzed by PLS-DA was shown in (a). The most significant differentially abundant taxa between CTL, CS and ABX groups were identified by LEfSe analysis (b). CTL-enriched taxa were indicated in blue, those in CS group were indicated in green and those in ABX group were indicated in red. Only taxa meeting the criteria for feature selection in LDA score (log10) > 4.0 with $P < 0.05$ were shown. A taxonomic cladogram representation of data was shown in panel (c). Red, ABX-enriched taxa; green, CS-enriched taxa; blue, taxa enriched in CTL group. The size of each dot is proportional to its relative abundance.



Supplementary Figure S10. Pg-LPS ameliorates COPD development in CS BALB/c mice. Pg-LPS was administered at 0.2 μg/mouse twice per week for 3 months in CS BALB/c mice. After *i.p.* treatment of Pg-LPS, the body weight change (a, b), lung histopathology (c), the mean linear intercept of lung tissue (d), lung functions analyses including FRC (e), FVC (f), Cchord (g), and FEV100/FVC (h) were shown. Except panel c, statistical analysis was performed using the Newman-Keuls multiple comparison post hoc one-way ANOVA analysis (n=5). ***, P<0.001.