Cell Reports, Volume *23*

Supplemental Information

Induction of Nitric-Oxide Metabolism in Enterocytes Alleviates Colitis and Inflammation-Associated Colon Cancer

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Supplementary Experimental Procedures

Colitis induction: Colitis was induced in *Vav1^{Cre}:Asl$^{f/f}$* mice and *CD11c^{Cre}:Asl$^{f/f}$* mice with 1.5% DSS and with 0.8% DSS in the *Villin^{Cre}:Asl$^{f/f}$* (wt/vol), (Dextran Sodium Sulfate, MP Biomedicals; molecular weight 36,000–50,000 Da). The powder was dissolved in the drinking water for 6 days, followed by 5 days of supplementing regular water (Wirtz et al., 2007). Each group consisted of eight to ten, 8-10 weeks old, male mice. Experiments were repeated at least three times.

Generation of BM chimeric mice. *Villin^{Cre}:Asl$^{f/f}$* or Asl$^{f/f}$ male mice aged 10–12 weeks were used as recipients. Mice were irradiated with a dose of 950 Gy and reconstituted via IV injection with 5×10^6 BM cells of either wt or *CX$_3$CR1^{Cre}:Il10ra $^{fl/fl}$* mice.

Arginine free diet: mice received arginine free diet (Envigo,TD09152 TEKLAD) since weaning and along the experiment.

Endoscopic evaluation of colitis: Colonoscopy was performed on days 7 and 11 of the experiment to monitor for severity of colitis. For the procedure, a high resolution mouse video endoscopic system was used, which consists of a miniature endoscope (scope 1.9 mm outer diameter), a xenon light source, a triple chip camera, and an air pump to achieve regulated inflation of the mouse bowel (Karl Storz, Tuttlingen, Germany). Mice were anesthetized by the administration of 100 mg/kg Ketamine/10 mg/kg Xylazine mixture IP, after which, the endoscope was introduced via the anus and the colon was carefully insufflated with an air pump before analysis of the colonic mucosa. The endoscopic procedure was viewed on a color monitor and digitally recorded on tape. Colitis was scored according to the Murine endoscopic index of colitis severity (MEICS), considering differences in the thickness of the bowel wall, changes in blood vessel integrity, mucosal surface, stool consistency or the presence of fibrin, scored each between 0 and 3. The cumulative score ranged from 0 (no signs of inflammation) to 15 (signs of severe inflammation) (Becker et al., 2006).

Drug administration: Mice were treated with 1% (wt/vol) DSS for 6 days, followed by administration of either arginine (Sigma-Aldrich, St. Louis, MO, catalogue number A8094) 1% (wt/vol) solution in drinking water, citrulline 1% (wt/vol) (L-citrulline, Chem-Impex International), $NaNO_2$ 100mg/kg (Sigma-Aldrich, St. Louis, MO, catalogue number S2252), or water as control for additional 6 days.

Fisetin was given by i.p. injection (1 mg/animal) in 30 µL of DMSO twice weekly. The control group was injected with 30 µL of DMSO twice weekly. Each group consisted of ten, 8 weeks old, male mice. Experiments were repeated three times.

Colitis associated cancer induction: 10- to 12 weeks old female mice, each group consisted of eight to ten mice, were injected with Azoxymethane (AOM) (Sigma) intraperitoneally at a dose of 12.5 mg/kg body weight. After 6 d, *Villin^{Cre}:Asl$^{f/f}$* mice were treated with 2% DSS (MP Biomedicals) (M.W. 36,000–50,000 Da) in the drinking water for 5 d, then followed by 16 d of regular water. This cycle was repeated twice. C57BL/6J.OlaHsd mice were treated with 1.5% DSS in the drinking water for 6 days followed by 15 d of administration of either fisetin (1mg in 30µl DMSO IP, twice, in 7 days interval) citrulline 1% (wt/vol) solution in drinking water, the combination of both treatments or only DMSO as a control (30µl DMSO IP, twice, in 7 days interval). This cycle was repeated twice. Colonoscopy was performed on days 40, 60 and 80 of the experiment. Tumorigenesis was evaluated according to previously described tumor scoring system (Becker et al., 2006).

Histopathology. On the day of sacrifice, colons were removed and their length was measured. Colon was fixed in 2.5% PFA solution overnight at 4°C, then embedded in paraffin, sectioned and stained with H&E. Tissues were examined in a blinded manner by a pathologist and scored on a 0–4 scale based on the parameters of inflammation severity(Hs et al., 1993). Histologic evaluation of *CX$_3$CR1^{Cre}:Il10ra $^{fl/fl}$* mice as previously described (Zigmond et al., 2014). Briefly, three segments of the colon (proximal colon, medial colon and distal colon-rectum) were given a score between 0-4 and the summation of these scores provided a total colonic disease score.

Immunohistochemistry: Four micrometer paraffin embedded tissue sections were deparaffinized and rehydrated. Endogenous peroxidase was blocked with three percent H2O2 in methanol. Sections undergoing for ALS staining were incubated in cold acetone at -20ºc for 7 minutes. For F4/80 and MAC-2 staining, we performed antigen retrieval in Tris-EDTA pH9 and 10 mM citric acid pH 6, for 10 min respectively, using a low boiling program in the microwave to break protein cross-links and unmask antigens. After pre-incubation with 20% normal horse serum and 0.2% Triton X-100 for 1 hour at RT, sections were incubated with the primary antibodies as follow; ASL (1:100, Abcam, ab97370, CA, USA); F4/80 (1:50, Serotec, Kidllington, UK); Mac-2 (1:400, Cedarlane, NC, USA); for fluorescent double-staining of ASL and CD11c we used mouse anti ASL (1:50 Santa Cruz

Biotechnology, Inc. sc-166787, TX, USA) and rabbit anti CD11c (1:50, ab52632) . All antibodies were diluted in PBS containing 2% normal horse serum and 0.2% Triton. Sections were incubated overnight at RT followed by 48h at 4°C. Sections were washed three times in PBS and incubated with secondary biotinylated IgG at RT for 1.5 hour, washed three times in PBS and incubated with avidin-biotin Complex (*elite* ABC kit, Vector Lab, CA, USA) at RT for additional 90 min followed by DAB (Sigma) reaction. For the fluorescent staining we used CY2 conjugated anti mouse and CY3 conjugated streptavidin (1:100 and 1;200 respectively, Jackson ImmunoResearch, West Grove, PA)
Tunnel staining was performed by using ApopTag kit detection according to manufacturer's instructions (Millipore, CA, USA). Stained sections were examined and photographed by a fluorescence or bright field microscope (Eclipse Ni-U; Nikon, Tokyo, Japan) equipped with Plan Fluor objectives (10; 20x; 40x) connected to a monochrome camera (DS-Qi1, Nikon).

***In vivo* intestinal permeability assay.** To assess barrier function was performed using an FITC-labeled dextran method. Food and water were withdrawn for 3h and mice were orally administrated with permeability tracer (80 mg/100 g body weight of FITC-labeled dextran, MW 4000; FD4, Sigma-Aldrich). Serum was collected retro-orbitally three hours later and fluorescence intensity was determined (excitation, 492 nm; emission, 525 nm; BioTek). FITC-dextran concentrations were determined using a standard curve generated by serial dilution of FITC-dextran.

Nitrite and nitrate concentrations in blood, colon: On the day of sacrifice, blood and 1cm of the distal colon were collected. Blood was separated by centrifuge (1500 RPM for 5 minutes) to plasma and red blood cells (RBC). Colon tissue was homogenized. Proteins in each sample were removed by centrifugation at 10,000g for 5 min following methanol precipitation (colon:methanol = 1:2 weight/volume, RBC:methanol = 1:1 volume/volume, 4 ^{o}C).
Nitrite concentration in the colon and the plasma was measured using a dedicated HPLC system (ENO-20; EiCom, Kyoto, Japan). This method is based on the separation of nitrite and nitrate by ion chromatography, followed by on-line reduction of nitrate to nitrite, post column derivatization with Griess reagent, and detection at 540 nm. Proteins in each sample were removed by centrifugation at 10,000g for 5 min following methanol precipitation (colon:methanol = 1:2 weight/volume, plasma:methanol = 1:1 volume/volume, 4°C).

Mouse colon epithelial and lamina propria isolation: To achieve cell purity, we used the 2 step protocol for isolation of different intestinal compartments (Berger et al., 2017; Elinav et al.,

2011); first we isolated intestinal epithelium and then, we isolated lamina propria CD45+ cells. The purity of different compartments was quality tested by staining for CD326 and CD45 markers respectively (King et al., 2012). Colon segment was flushed with cold PBS, longitudinally cut open and rinsed to remove residual luminal contents. Tissue was cut into 3- to 5-mm pieces and incubated in HBSS medium containing EDTA 0.5M and Hepes10mM at 37°C for 20 min, shaken at 180 rpm, to separate epithelial fraction. Supernatant was centrifuged at 4°C at 500 g for 10 minutes. Recovered tissue was digested in HBSS medium with 2% fetal bovine serum (FBS), 0.05% collagenase II and 0.05% DNase I (Sigma) for 40 min. at 37°C, shaken at 180 rpm. LP cells were filtered through 65μm mesh and centrifuged at 4°C at 500 g for 10 minutes.

Flow cytometry: We prepared single cell suspensions from mouse colon by enzymatic digestion and analyzed by polychromatic flow cytometry. Samples were stained with conjugated antibodies or matching isotype controls according to manufacturer's instructions. Data were acquired on an LSRII (BD Biosciences) or FACSCalibur instrument (Becton-Dickinson) with CellQuest software, with a MacsQuant instrument (Miltenyi, Bergisch Gladbach, Germany), and analyzed using BD FACSDiva 6 or FlowJo software (version 7.6.5, or version vX.0.7 Tree Star Inc) or MacsQuant. Colon cells were stained for 30 min at 4 °C in flow cytometry buffer (PBS, 10% FCS and 0.02% azide). For antigens that require intracellular staining (Asl, iNOS and S-nitrosocystein), cell surface staining was followed by cell fixation and permeabilization with the Cytofix/Cytoperm kit (BD Biosciences) according to the manufacturer's instructions. For staining, we used a lineage cocktail of antibodies anti-CD45-APC, Anti-CD64-PE, Anti – CD11c-PB, Anti-326-APC cy7, Anti CD31-PE cy7 (all from BioLegend). After staining for cell surface antigens, intracellular NO was detected by incubation of cells for 15 min at 37 °C with 10 μM DAF-FM diacetate followed by extensive washing, according to the manufacturer's instructions (Molecular Probes). Staining without addition of the DAF-FM probe was considered to be the baseline for gating the positive population. Asl was detected using anti-Asl- ALEXA FLUOR® 488 (BD Biosciences), iNOS using anti-iNOS followed by anti-rat 488 (Jackson ImmunoResearch), and nitrosylation using anti-S-nitrosocystein (from abcam) followed by anti-mouse 488 (Jackson ImmunoResearch).

Macrophage elicitation and stimulation: Thioglycollate elicited macrophages were isolated by peritoneal lavage from five, 8 weeks old mice, in each group. Mice were i.p. injected with 1ml sterile thioglycollate broth and four days after, peritoneal macrophages were isolated following a previously described method by Zhang et al. (Zhang et al., 2008).

Equal numbers of peritoneal cells were allowed to adhere to cell culture dishes, two plates for each extract, for four hours. Non-adhered cells were removed and adherent cells were washed twice. Cells were cultured in RPMI 1640 arginine free medium containing 20% fetal calf serum (heat inactivated), 1% Penicillin/Streptomycin and 2% L-Glutamine (all from Biological Industries). For each extract, one plate was stimulated with LPS ultrapure (1µg/ml) (Lipopolysaccharide, Ultra Pure, Salmonella, Sigma-Aldrich), for a total of 18h or 36 h while the other plate served as control. Medium was taken for NO analysis and glucose and lactate levels using NOVA.

Assessment of plasma arginine concentrations: Blood was collected at the time of sacrifice via cardiac puncture, placed in heparin coated tube and kept on ice. The samples were centrifuged for 5 min at 7,000 rpm. The supernatant was removed, aliquoted, snap frozen, and stored at -80°C until used for amino acid analysis. Analysis was conducted by analytical laboratory services (AMINOLAB LTD).

Assessment of colon epithelial cells arginine and polyamines concentrations: The analysis was performed according to a previously described protocol (Gray and Plumb, 2014) with slight modifications. Briefly, equal number of isolated colon epithelial cells (~$3*10^6$) were incubated in a phosphoric:acetic acid buffer supplemented with 1:50 (v/v; 10 µM) of the following internal standards: hexamethylene diamine and 13C6-arginine (Cambridge Isotope Laboratories). Following 30 min at 40C, cell lysates went through three freeze-thaw cycles using liquid nitrogen.

For the determination of polyamines and amino acids, equal protein 10-µl samples (12 µg) were added to 70 µL of borate buffer (200 mM, pH 8.8 at 25°C) and mixed. Then, 20 µl of Aqc reagent (10 mM dissolved in 100% ACN) were added and immediately mixed. Aqc reagent was prepared following the procedure described (Cohen and Michaud, 1993). For the determination of proline and arginine, samples were first diluted with the borate buffer 1:10, and then 10-µl aliquots were reacted with Aqc. For derivatization, the samples were heated at 55°C for 10

minutes, centrifuged at maximum speed for three min and then filtered through a 0.2-µm PTFE filter (Millex-LG, Millipore) to HPLC vials containing inserts.

The LC-MS/MS instrument consisted of Acquity I-class UPLC system and Xevo TQ-S triple quadrupole mass spectrometer (Waters). Chromatographic conditions were used as described in (Gray and Plumb, 2014). Mass detection was carried out using electrospray ionization in the positive mode. Argon was used as the collision gas with a flow of 0.1 ml/min. The capillary voltage was set to 3.0 kV, source temperature - 150°C, desolvation temperature - 650°C, desolvation gas flow - 800 L/min, cone voltage 20V. Analytics were detected by monitoring of fragment ion 171 m/z produced from corresponding precursor ions using parameters described in (Zwighaft et al., 2015) for polyamines, and in (Gray and Plumb, 2014) for amino acids, with exception that collision energy 20eV was used for regular and labeled 13C6 arginine.

The concentrations were calculated as the response ratio between IS and the analyte using calibration curves of the corresponding compounds. Data processing was performed with TargetLynx software.

ELISA: For cytokine production measurements, colon tissue single cell suspension was assayed for cytokine levels using a mouse cytokine quantibody array (RayBiotech), according to the manufacturer's instructions. Nitrosilation level was measured using 3-Nitrotyrosine ELISA Kit (abcam), according to the manufacturer's instructions.

Complete blood count: Blood was collected at the time of sacrifice via cardiac puncture, placed in EDTA coated tube. Diagnostic Veterinary Pathology Services (PathoVet).

DNA extraction from blood: QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) (Qiagen, Valencia, CA), DNA was isolated from 200 µl of blood according to manufacturer's instruction, in the final step DNA was eluted in 200 µL of buffer AE. The DNA eluate was stored at - 20°C until use in qPCR analysis.

Western blot: Western blotting was performed as previously described (Becker systems (Perkin Elmer) and a fluorescence microscope (Olympus, et al., 2003). In some experiments, culture supernatants were used and concentrated by acetone precipitation. Equal amounts of extract (30 or 50 µg) were added to 10 µl electrophoresis sample buffer. After boiling, the proteins were separated by 10% SDS - PAGE, then transferred to nitrocellulose membranes and detected with a specific antibody Anti-Argininosuccinate Lyase (ab97370) and the ECL Western blotting analysis system (Amersham).

Small molecule screen analysis: MANTRA (Iorio et al., 2010; Napolitano et al., 2016) is built upon the Connectivity Map dataset (Lamb et al., 2006) where 1,309 small molecules have been profiled by Affymetrix microarrays on 5 different cell lines at different dosages for a total of 7,000 gene expression profiles (GEPs). MANTRA collapses the 7,000 GEPs into 1,309 Prototype Ranked Lists (PRLs), i.e. one for each small molecule. PRLs represent a consensus rank of differentially expressed genes following treatment with the same drug across multiple cell lines and different dosages. In order to identify the drugs upregulating ASS1 and ASL, we performed a Gene Set Enrichment Analysis (GSEA) on the PRLs for each of 1,309 drugs using the MANTRA online web tool (http://mantra.tigem.it).

MRI: Prior to MRI imaging, animals were anesthetized using a mix of Medetomidine/Ketamine. The mouse colon was cleaned using warm water and perfluorinated oil was introduced into the colon via a rectal catheter. MRI experiments were performed on a 9.4T Bruker BioSpec system using a quadrature volume coil with 35-mm inner diameter. T2 maps were acquired using a multi-slice, spin-echo imaging sequence with the following parameters: repetition delay (TR) of 3000 ms, 16 time-echo increments (linearly spaced from 10 to 160 ms), matrix dimension of 256 x 128 and two averages, corresponding to an image acquisition time of 12 min 48 sec. Fourteen continuous slices with slice thickness of 1.0 mm were acquired with a field of view of 3.25 x 2.5 cm2. Quantitative T2-maps were generated from multi-echo, T2-weighted images using an in-house Matlab program. The average T2 of the colon was calculated for each slice. Differences in the mean T2 across all of the images slices were compared using a T-test statistic test.

Caco-2 preparation. Caco-2 cells were cultured in Dulbecco's modified Eagle's minimum essential medium (DMEM, pH 7.4) supplemented with 25 mM glucose, 10% inactivated fetal bovine serum (FBS), 1% penicillinstreptomycin and 1% non-essential amino acid solution. Cells were maintained at 37˚C in a humidified 5% CO2 atmosphere.

Determinations of transepithelial electrical resistance (TEER). Caco-2 cells were grown in the same medium and plated at 1x105 cells/100 µl on Millicell- cell culture inserts (Millipore, Bedford, MA). Cellular TEERs were measured with an electrical resistance system, Millicell-ERS-2 (Merck). Cells with stable TEER readings >500 Ωcm^2 were used. Medium was replaced with either medium containing citrullne 1% and fisetin 100µM dissolved in DMSO or control medium containing DMSO. After 24 hr, 1% DSS (MP Biomedicals; molecular weight 36,000–

50,000 Da) was exposed to apical sides of Caco-2 cell monolayers. Analyses were performed in >5 replicates.

16S rRNA Analysis: Stool samples of 12 weeks old *Asl*$^{f/f}$ and *Villin*^{Cre}*:Asl*$^{f/f}$ mice on arginine free diet since weaning, and *Asl*$^{f/f}$ mice on standard diet were collected and froze in liquid nitrogen. Samples were processed for DNA isolation using MoBio (PowerSoil kit) according to the manufacturer's instructions. The purified DNA from feces was used for PCR amplification and sequencing of the bacterial 16S rRNA gene. Amplicons of ~380 base pairs spanning the variable region 3-4 (V3-4) of the 16S rRNA gene were generated by using designated primers. The PCR products were subsequently pooled in an equimolar ratio, purified (PCR clean kit, Promega), and used for Illumina MiSeq sequencing. Reads were processed using the QIIME (quantitative insights into microbial ecology) analysis pipeline as described (Elinav et al., 2011) version 1.8. Paired-end joined sequences were grouped into operational taxonomic units (OTUs) using the UCLUST algorithm and the GreenGenes database (DeSantis et al., 2006). Sequences with distance-based similarity of 97% or greater over median sequence length of 353 bp were assigned to the same OTU. Analysis was performed at each taxonomical level (phylum to genus and specie level if possible) separately. For each taxon, statistical tests were performed between the different groups. P values was FDR-corrected for multiple hypothesis testing.

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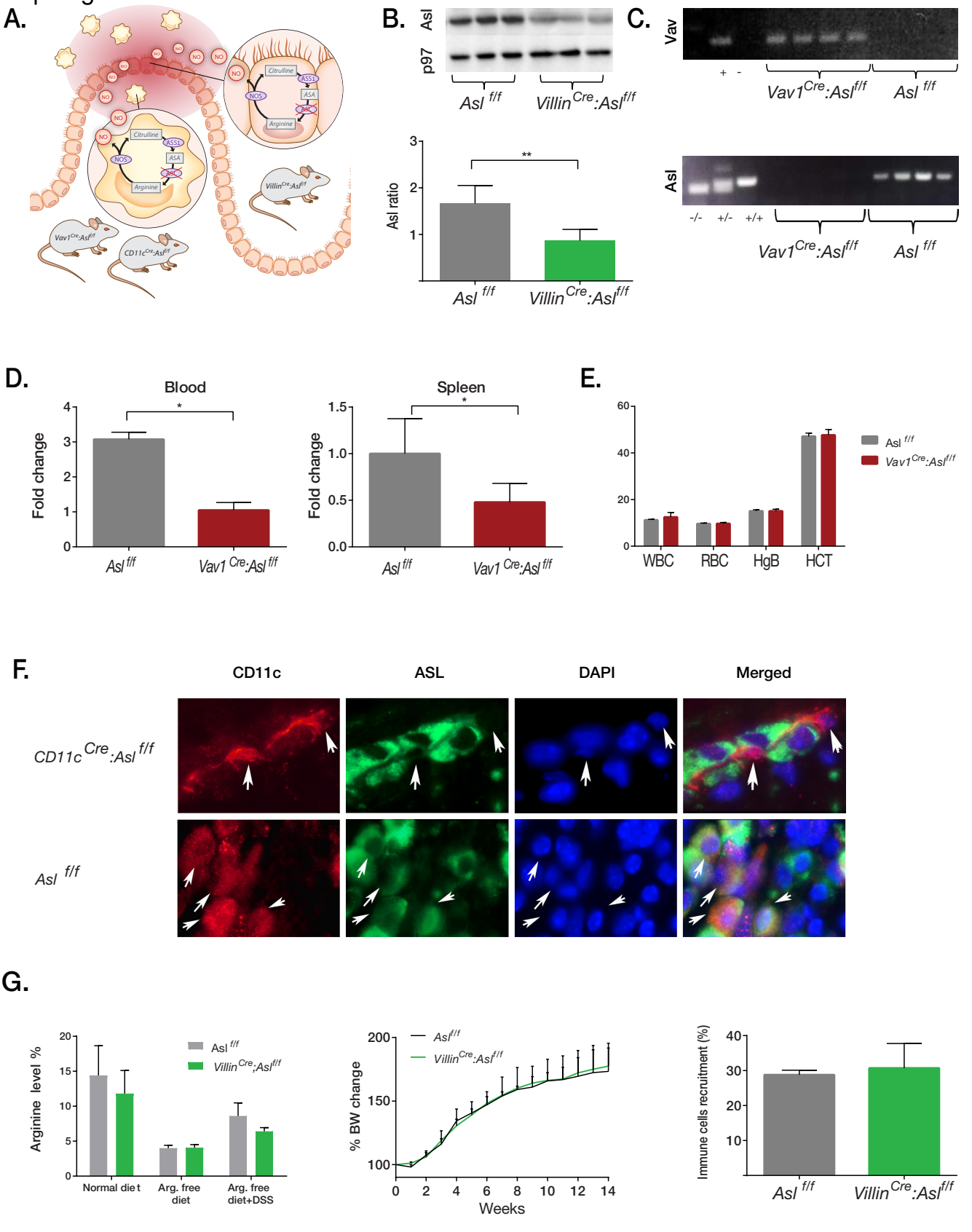
Sup. Figure 1


A.
B.
Asl
p97
Asl f/f
Villin Cre:Asl f/f
Asl ratio
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C.
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Vav1 Cre:Asl f/f
Asl f/f
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D.
Blood
Spleen
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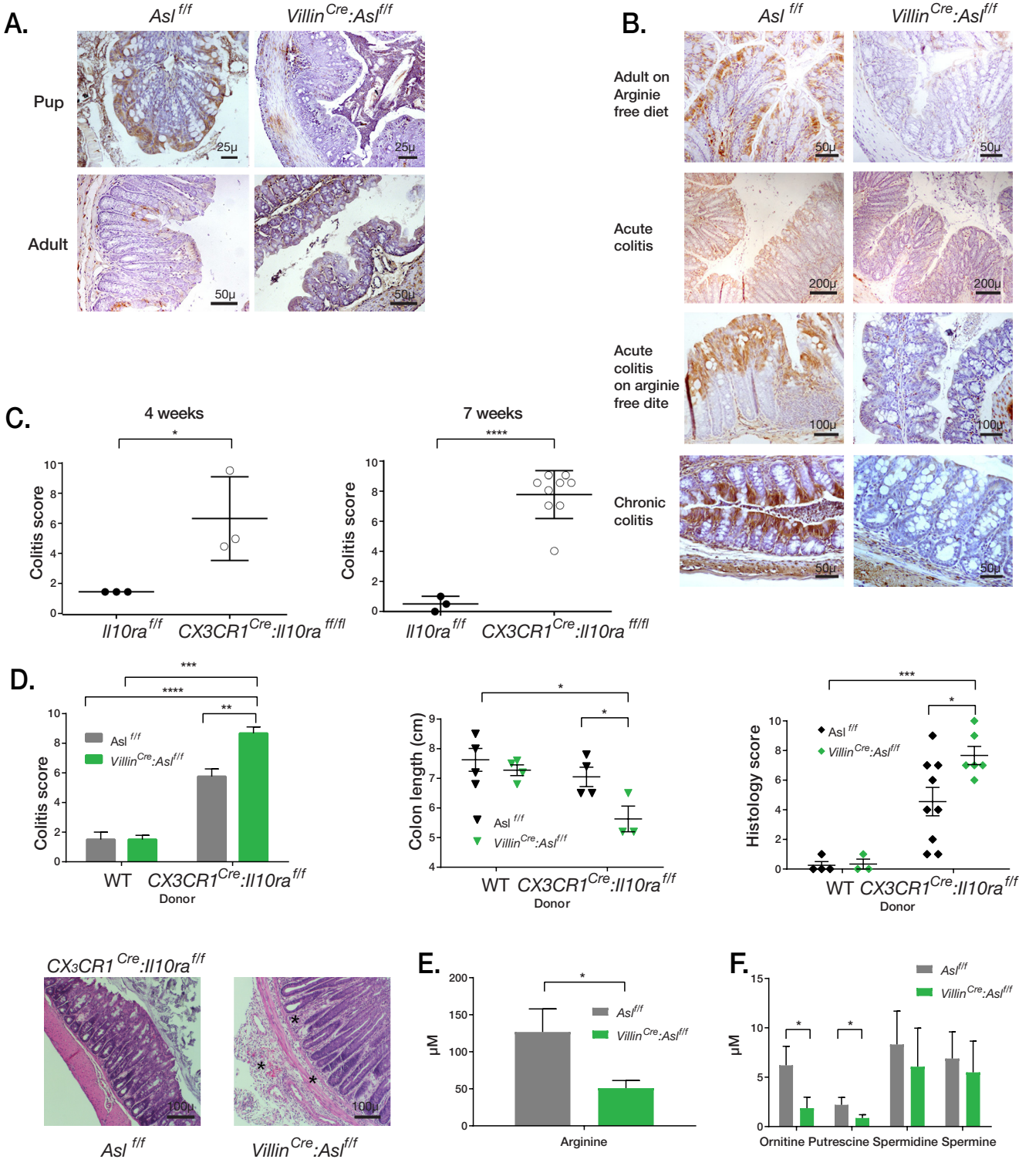
E.
WBC
RBC
HgB
HCT
F.
CD11c
ASL
DAPI
Merged
CD11c Cre:Asl f/f
Asl f/f
G.
Arginine level %
Normal diet
Arg. free diet
Arg. free diet+DSS
% BW change
Weeks
Immune cells recruitment (%)

Supplementary Figure 1- Generation of ASL CKO mice and a genetic colitis model. Related to figures 1,2,3 and 4 **(A)** An illustration of the experimental model using mice with deletion of Asl in the hematopoietic system - Vav1Cre: Aslf/f, and CD11cCre:Aslf/f and in mice with deletion of Asl in the epithelial cells - VillinCre:Aslf/f. The magnified cells in circles represent the CKO in enterocytes by VillinCre:Aslf/f (upper right) and the CKO in immune cells in general by VavCre+/- and specifically in macrophages by CD11cCre+/- (lower left). **(B)** A western blot on whole intestinal homogenate showing decreased ASL protein expression in intestine in of neonatal VillinCre:Aslf/f mice as compared to controls. **(C)** A PCR gel of DNA from blood shows expression of Vav Cre but not of Asl in the Vav1Cre: Aslf/f mice; (-/-) complete KO of Asl, (+/-) heterozygous Asl KO and (+/+) wild type Asl expression, (n= 4 in both groups). **(D)** RT PCR showing decreased Asl expression in Vav1Cre: Aslf/f mice' blood (left panel) and spleen (right panel), as normalized to levels of the housekeeping gene Hypoxanthine Phosphoribosyltransferase (HPRT), (n= 3 in each group). **(E)** A complete blood count shows no differences between the Vav1Cre: Aslf/f and control groups. WBC; white blood cells, RBC; red blood cells, HgB; hemoglobin, HCT; hematocrit, (n=6 in each group). **(F)** Immunohistochemistry staining for ASL in CD11c cells showing ASL expression in Aslf/f mice (merged panel), but not in CD11cCre: Aslf/f. Arrows indicating CD11c expression. In red- CD11c, green- ASL, blue – DAPI. **(G)** Plasma arginine levels are similar in VillinCre:Aslf/f and in control mice fed for 10 weeks after weaning with a normal or arginine free diet at baseline or after induction of colitis,(left panel) (n>3 in each group). Arginine deficient diet did not cause growth differences between control and VillinCre:Aslf/f measured over 14 weeks post weaning (middle panel) or in recruitment of CD45+ immune cells to the intestine as determined by FACS (right panel). Statistical analyses were performed using one-way ANOVA, two-way ANOVA, repeated-measures ANOVA or Student's t-test. ; *P <0.05, ** P <0.005.

Sup. Figure 2


A.
Asl f/f
Villin Cre:Asl f/f
Pup
Adult
25µ
50µ
B.
Asl f/f
Villin Cre:Asl f/f
Adult on Arginie free diet
Acute colitis
Acute colitis on arginie free dite
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50µ
200µ
100µ
C.
4 weeks
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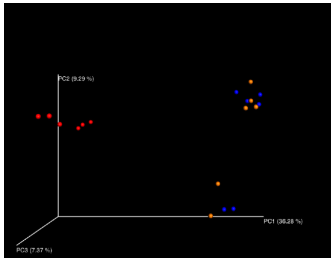
Colitis score
Il10ra f/f
CX3CR1 Cre:Il10ra ff/fl
D.
Asl f/f
Villin Cre:Asl f/f
WT
CX3CR1 Cre:Il10ra f/f
Donor
Colitis score
Colon length (cm)
Histology score
CX3CR1 Cre:Il10ra f/f
Asl f/f
Villin Cre:Asl f/f
E.
µM
Arginine
F.
Ornitine Putrescine Spermidine Spermine

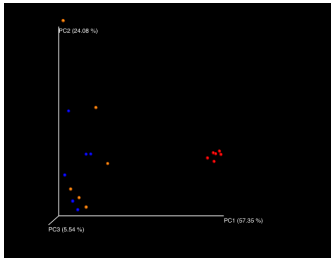
Supplementary Figure 2 - ASL expression levels in enterocytes is state specific. Related to figures 1 and 2. **(A)**. Immunohistochemistry staining for ASS1 showing that ASL expression in the intestine of 10 days old pups decreases in the intestine of adult mice. **(B)** Immunohistochemistry staining shows increased ASL expression in the intestine of control mice when they are fed with arginine free diet for 10 weeks after weaning. Whereas induction of acute colitis per se does not upregulate ASL in the control mice enterocytes, an increased expression is seen during colitis when fed with arginine-free diet. An increase in ASL in control mice enterocytes was seen also after induction of the chronic colitis model in which the colitis was induced for 2 months **(C)** Lethally irradiated wild type animals that received a bone marrow (BM) graft from CX3CR1Cre:Il10rafl/fl developed colitis as indicated by colitis score at 4 weeks and 7 weeks after BM implantation, as compared to control chimeras. **(D)** Colitis was induced by bone marrow (BM) transplantation from wildtype (WT) or CX3CR1Cre:Il10ra fl/fl donors, to lethally irradiated VillinCre:Aslf/f and to control Aslf/f mice. While no colitis signs were detected in mice receiving WT BM, VillinCre:Aslf/f mice transplanted with CX3CR1Cre:Il10ra fl/fl BM, had increased severity of colitis compared to control mice, as demonstrated by a higher endoscopic colitis score 5 weeks after BM transplantation (left panel) (n>3 in each group); shorter colon length (middle panel), and by a higher histologic score due to increased infiltration, edema and ulcers (right panels), representative colon cross section stained with H&E showing extensive edema and cellular infiltration in the VillinCre:Aslf/f mice compare to controls, (left lower panel) (n>3 in each group). Asterisks indicate areas with edema and increased cellular infiltration. **(E)** Liquid chromatography–mass spectrometry (LC/MS) measurement of arginine shows significantly reduced levels in the enterocytes of VillinCre:Aslf/f mice as compared to arginine levels in control mice on day 11 after colitis induction **(F)** Following colitis induction, polyamine measurements using LC/MS show significantly reduced levels of ornithine and putrescine in enterocytes of VillinCre:Aslf/f mice as compared to control mice, while no differences were observed in the spermidine and spermine levels. Statistical analyses were performed using one-way ANOVA, two-way ANOVA, repeated-measures ANOVA or Student's t-test; *P <0.05, ** P <0.005, ***P <0.0005, ****P <0.00005.

Sup. Figure 3

A.



Weighted



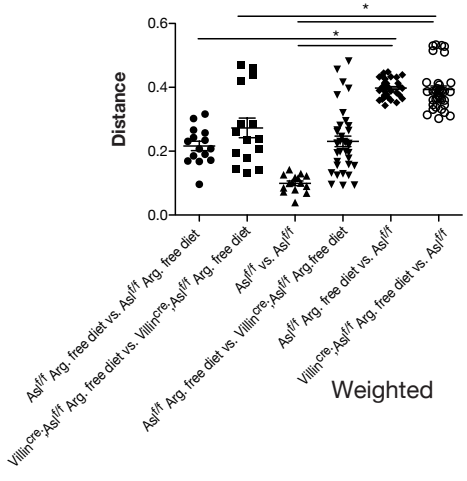
Unweighted

$Asl^{f/f}$

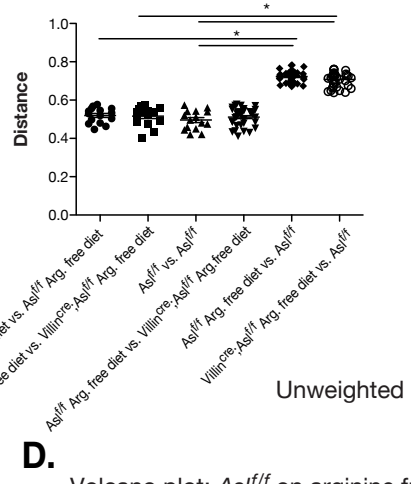
$Asl^{f/f}$ Arg.free diet

$Villin^{cre}$;$Asl^{f/f}$ Arg. free diet

B.

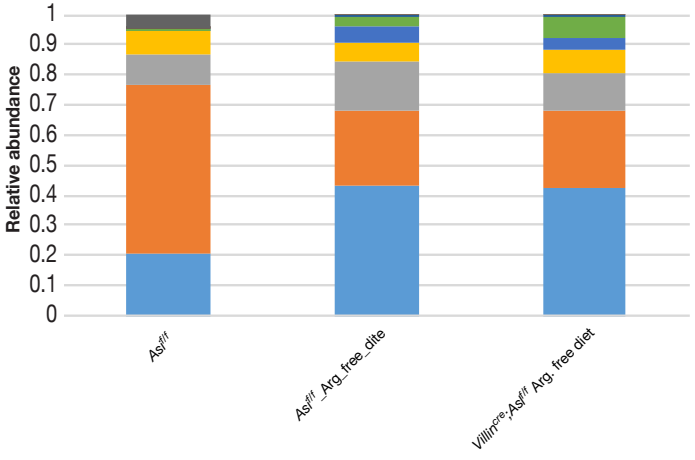


Weighted



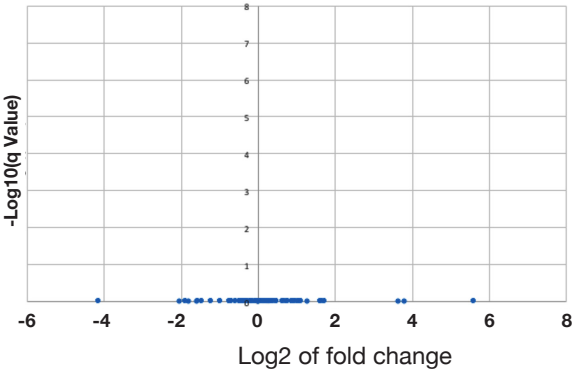
Unweighted

C.



D.

Volcano plot: $Asl^{f/f}$ on arginine free diet vs. $Villin^{Cre}$:$Asl^{f/f}$ on arginine free diet



k__Bacteria;p__Firmicutes

k__Bacteria;p__Bacteroidetes

k__Bacteria;p__Proteobacteria

k__Bacteria;p__Verrucomicrobia

k__Bacteria;p__Actinobacteria

k__Bacteria;p__Deferribacteres

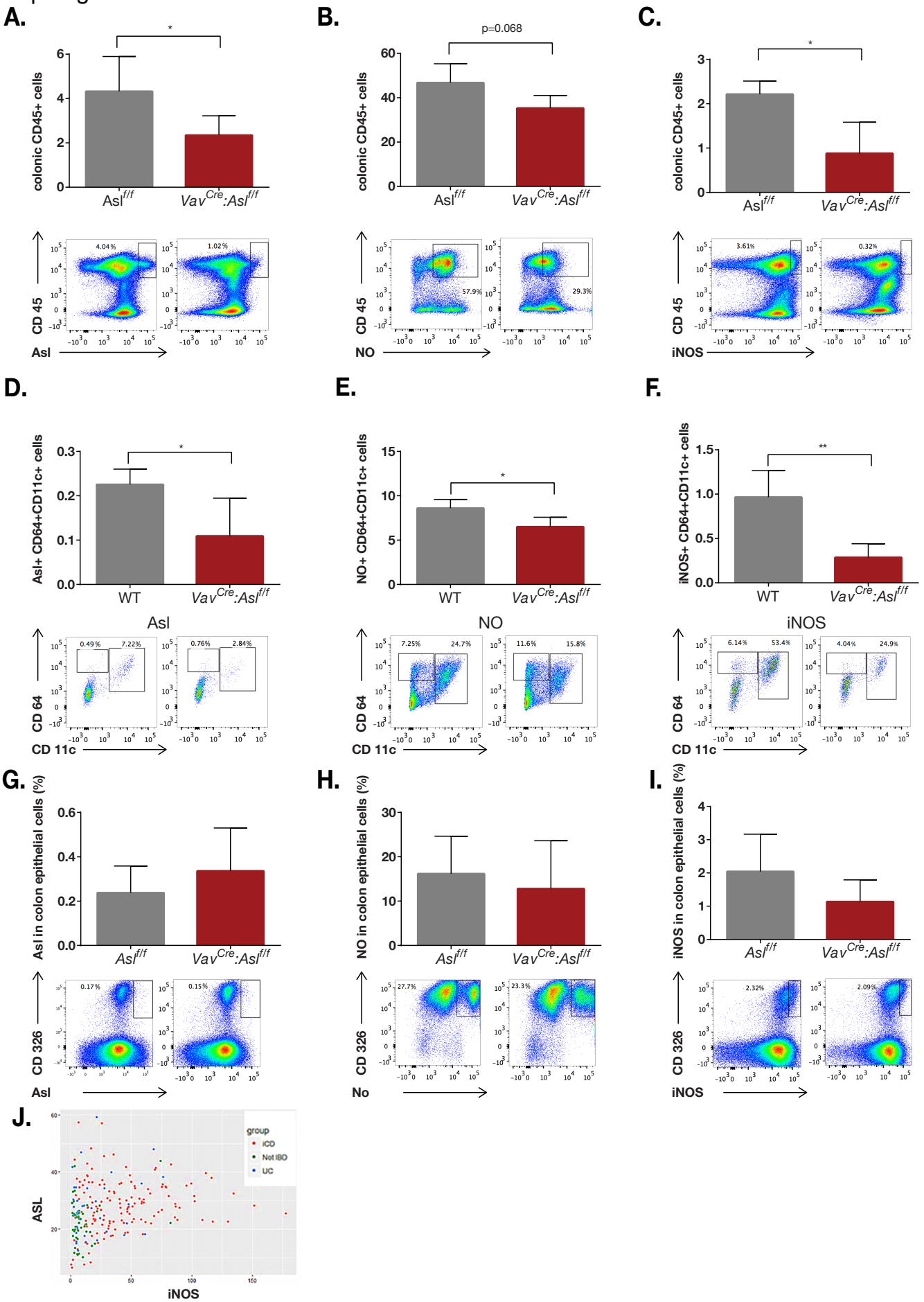
Unassigned;Other

k__Bacteria;p__Tenericutes

k__Bacteria;p__Cyanobacteria

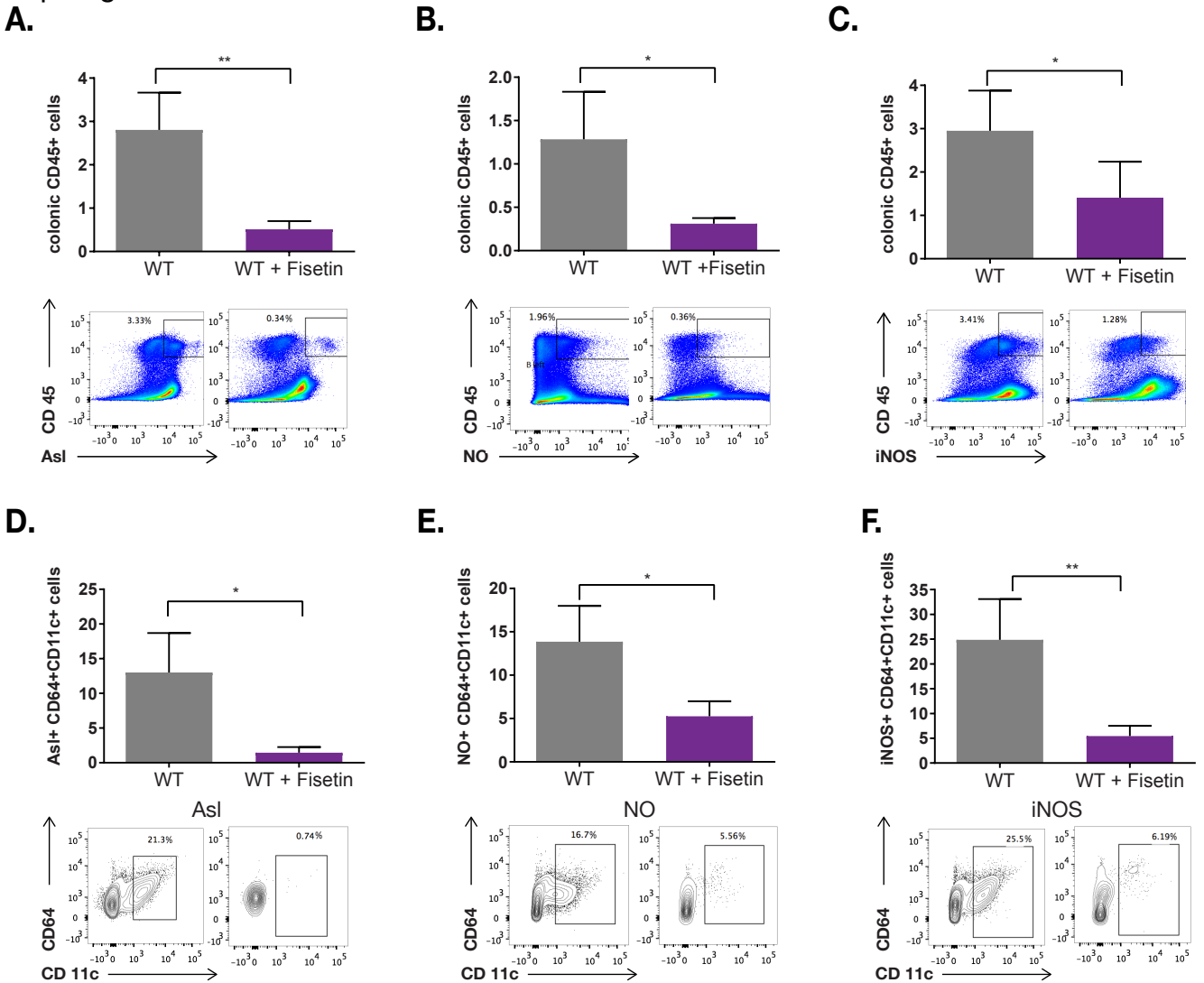
Supplementary figure 3: ASL expression levels in the enterocytes does not affect the microbiome. Related to figures 1 and 2. Stool samples of 12 weeks old Aslf/f and VillinCre:Aslf/f mice on arginine free diet since weaning, and of Aslf/f mice on standard diet, were collected for 16S rRNA sequencing analyses. No separation between Aslf/f and VillinCre:Aslf/f mice on arginine free diet are seen, as shown by 3D weighted and un-weighted Principal coordinates analysis (PCoA) of UniFrac distances of 16S rRNA sequencing **(A)**, by quantification of the UniFrac distances of 3D weighted and un-weighted Principal coordinates analysis (PCoA) of 16S rRNA, by 1-way ANOVA with Bonferroni posttest **(B)**, by the averages of Phylum bacterial relative abundance in stool samples **(C)**, and by Volcano plot for bacteria taxa abundance **(D)**. N=6 in each group, * $P<0.05$.

Sup. Figure 4


A.
B.
C.
colonic CD45+ cells
Asl^f/f
Vav^Cre:Asl^f/f
p=0.068
4.04%
1.02%
57.9%
29.3%
3.61%
0.32%
CD 45
Asl
NO
iNOS
D.
E.
F.
Asl+ CD64+CD11c+ cells
NO+ CD64+CD11c+ cells
iNOS+ CD64+CD11c+ cells
WT
Asl
NO
iNOS
0.49%
7.22%
0.76%
2.84%
7.25%
24.7%
11.6%
15.8%
6.14%
53.4%
4.04%
24.9%
CD 64
CD 11c
G.
H.
I.
Asl in colon epithelial cells (%)
NO in colon epithelial cells (%)
iNOS in colon epithelial cells (%)
0.17%
0.15%
27.7%
23.3%
2.32%
2.09%
CD 326
No
J.
ASL
iNOS
group
iCD
Not IBD
UC

Supplementary Figure 4- Cell specific ASL deficiency correlates with decreased NO production. Related to figure 3 and 4. FACS analysis of colon cells from Vav1Cre: Aslf/f and from control Aslf/f mice, after colitis induction by DSS shows a significant increase in the levels of ASL, NO and iNOS in CD45+ colon immune cells of the control group but not in the Vav1Cre: Aslf/f mice **(A-C)**, as well as in the CD64+, CD11c+ macrophages. **(D-F)**, however no differences in these parameters are seen in CD326 colon enterocytes **(G-I)**, ($n \geq 4$ in each group). Statistical analyses were performed using Student's t-test; *P <0.05, ** P <0.005. **(J)** ASL expression correlates with iNOS in colon of patients with and without IBD, analysis of GEO dataset- GSE57945 ID: 200057945, (R=0.27; pV= 2.612e-05).

Sup. Figure 5


A.
B.
C.
D.
E.
F.
colonic CD45+ cells
WT
WT + Fisetin
WT +Fisetin
CD 45
AsI
NO
iNOS
3.33%
0.34%
1.96%
0.36%
3.41%
1.28%
AsI+ CD64+CD11c+ cells
NO+ CD64+CD11c+ cells
iNOS+ CD64+CD11c+ cells
CD64
CD 11c
21.3%
0.74%
16.7%
5.56%
25.5%
6.19%

Supplementary Figure 5 - Fisetin does not increase NO levels in intestinal immune cells. Related to figure 6. FACS analysis of colon cells from mice treated with fisetin vs control after colitis induction shows decrease in ASL, NO and iNOS expression levels in CD45+ immune system cells after fisetin treatment **(A-C)**, as well as in the CD64+, CD11c+ macrophages **(D-F)**, (n=4 in each group). Statistical analyses were performed using Student's t-test; *P <0.05, ** P <0.005.

Table 1

Gene	Up	Down
ASL	alsterpaullone	Prestwick-1083
	copper_sulfate	baclofen
	dexamethasone	butirosin
	fisetin	chlorpropamide
		clofazimine
		doxepin
		isosorbide
		pinacidil
		pyridoxine
		thiocolchicoside
		trazodone
ASS1	azacitidine	cefotaxime
	fisetin	celecoxib
	staurosporine	

Supplementary Table 1: FDA approved small molecules that affect ASL and ASS1 expression levels. Related to figure 6 and 7. The table summarizes all FDA approved small molecules that upregulate or downregulate ASL and ASS1 expression levels in response to FDA approved small molecular screen.