**Supplemental Methods**

**Bone marrow preparation**
Bone marrow was harvested by flushing trimmed tibiae with approximately 5 ml per bone of Hank’s buffered saline solution (Gibco-Life Technologies) with 2% fetal bovine serum (Gibco-ThermoFisher) and 1% HEPES buffer (Gibco-ThermoFisher). The bone marrow suspension was filtered through a 40-um nylon cell strainer (Falcon-Corning). Cells were counted on a Cellometer (Nexcelcom) at 1:2 dilution in AOPI stain. Bone marrow cells were stained and analyzed on a LSRII flow cytometer (BD Biosciences).

**Staining for flow cytometry**
Cells were stained with antibody mixes at concentration 1:100 for each surface marker in Hank’s buffered saline solution with 2% fetal bovine serum and 1% HEPES buffer. Multicolor flow cytometry was performed on LSRII cell analyzers (BD Biosciences). Analysis of flow data was performed using FlowJo (FlowJo, LLC).

**Cell markers used for flow cytometry identification of cell populations:**

| Hematopoietic cells in bone marrow | Markers | Lineage combination |
|-----------------------------------|---------|---------------------|
| LSK                               | Lin^Sca1^cKit^+ | Gr1^+B220^+CD4^+CD8^+Ter119^+ |
| Hematopoietic stem cells          | LSK CD150^+CD48^+Flk2^+CD34^- | Gr1^+B220^+CD4^+CD8^+Ter119^+ |
| Multipotent progenitors 1 (MPP1)   | LSK CD150^+CD48^+Flk2^+CD34^- | Gr1^+B220^+CD4^+CD8^+Ter119^+ |
| Multipotent progenitors 2 (MPP2)   | LSK CD150^+CD48^- | Gr1^+B220^+CD4^+CD8^+Ter119^+ |
| Multipotent progenitors 3 (MPP3)   | LSK CD150^+CD48^+Flk2^+CD34^- | Gr1^+B220^+CD4^+CD8^+Ter119^+ |
| Multipotent progenitors 4 (MPP4)   | LSK CD150^+CD48^+Flk2^+CD34^- | Gr1^+B220^+CD4^+CD8^+Ter119^+ |
| Granulocytes                      | CD4^+CD8^-B220^-CD11b^-Gr1^-F4/80^-SSC^mid | N/A |

**Antibody clones and fluorophore conjugates used for flow cytometry:**

| Biomarker       | Clone   | Conjugate   | Manufacturer |
|-----------------|---------|-------------|--------------|
| B220 (CD45R)    | RA3-6B2 | eFluor-450  | eBioscience  |
|                 |         | FITC        |              |
|                 |         | PECy5       |              |
|                 |         | PECy7       |              |
| CD4             | GK1.5   | eFluor-450  | eBioscience  |
|                 |         | FITC        |              |
|                 |         | PE          |              |
|                 |         | PECy5       |              |
| CD8             | 53-6.7  | eFluor-540  | eBioscience  |
|                 |         | FITC        |              |
|                 |         | PECy5       |              |
| CD11b (Mac1)    | M1/70   | PECy7       | eBioscience  |
| CD34            | RAM34   | FITC        | eBioscience  |
| CD45.1          | A20     | PacBlue     | BioLegend    |
| CD45.2          | 104     | BV605       | BioLegend    |
| CD48            | HM48-1  | APC         | eBioscience  |
| CD117 (cKit)    | 2B8     | APC-eFluor780 | eBioscience |
| CD135 (Flk2)    | A2F10   | PE          | eBioscience  |
| CD150           | mShad150| APC         | eBioscience  |
|                 |         | PECy7       |              |
| F4/80           | BM8     | FITC        | eBioscience  |
| Gr1 (Ly6C-G)    | RB6-8C5 | APC         | eBioscience  |
|                 |         | PECy5       |              |
|                 |         | PECy7       |              |
| Sca1 (Ly6A/E)   | D7      | Pacific blue| BioLegend    |
Desaminotyrosine treatment
Antibiotic-treated mice were treated with DAT (20 mmol) by oral gavage every 2-3 days on days 7 to 14 of antibiotic treatment before analysis on day 14.

16s rRNA qPCR
Fecal pellets were collected from 3 mice per group and stored at -80 degrees Celsius. SYBR green (Biorad) quantitative PCR for 16S was performed with primers for 16s rRNA gene on DNA extracted from mouse fecal pellets using the DNeasy PowerSoil Kit (Qiagen) according to manufacturer’s protocol. A plasmid containing the 16s rRNA gene from Blautia was used to produce the standard curve for calculating gene copy number. qPCR was performed with the Roche Lightcycler 96 (Roche).

Quantitative PCR primers

| Gene  | Forward primer         | Reverse primer         | Source                       |
|-------|------------------------|------------------------|------------------------------|
| Ifitm3 | TTCAGTGCTGCCTTTGCTC | CTTTGATTCTTTGCTAGTTTTGGG | Melody et al., 2016, JEM     |
| lrf2 | AGAAGAGAGAGTTAACATCAAGCA | TTATAGCTGAAGTCAAGGCCGATACT | Masumi et al., 2009, FEBS Letters |
| lsg15 | GGAACGAAAGGGGCCACAGCA | CCTCCATGGGCTTCCCCCTGA | Dai et al., 2011, Virology Journal |
| Stat1 | CTGAATTTTCCCTCTGGG | TCCCCTACAGATGTCCATGAT | Shaul et al., 2010, Diabetes |
| 18s  | GTAACCGGTAGACCCCAT | CCATCCAATCGGATAGCG | Clifford et al., 2012, PLOS ONE |
| 16s  | ACTCCTACGGGAGGCAGCAGT | TATTACCGCGGCTGCTGGC |                             |

Fecal pellet collection for metabolomics
Mice were restrained by hand and an individual fecal pellet was directed into a 1.5 ml tube. The fecal pellet was immediately snap frozen in a dry ice/ethanol bath and stored at -80°C.

Serum collection for metabolomics
Blood was collected from mice via cardiac puncture and transferred to a BD Microtainer SST (BD). The tubes were spun down at 1000 xg for 30 minutes and the collected serum was stored at -80°C.

Complete blood counts
Blood was collected with a cardiac puncture with a 25 5/8G needle and syringe or retro-orbitally with heparinized Microhematocrit capillary tubes (Fisherbrand) and blood was analyzed using a scil Vet abc hematology analyzer (scil animal care company) or Advia 120 automated hematology system (Siemens).

Polar metabolite profiling by LC/IC-MS
To determine the relative abundance of polar metabolites in mouse stool samples, extracts were prepared and analyzed by ultra-high resolution mass spectrometry (HRMS). Mouse fecal pellets were snap frozen with liquid nitrogen, pre-pulverized, then homogenized in 1 mL ice-cold 80/20 (v/v) methanol/water using a Precellys Tissue Homogenizer. Metabolite extracts were centrifuged at 17,000 g for 5 min at 4°C, and supernatants were transferred to clean tubes, followed by evaporation to dryness under nitrogen.

To determine the relative abundance of polar metabolites in mouse serum samples, extracts were prepared and analyzed by ultra-high resolution mass spectrometry (HRMS). Mouse serum was thawed on ice, then 1 mL ice-cold 80/20 (v/v) methanol/water was added to 20 μL of mouse serum sample for polar metabolite extraction. Samples were vortexed for 2 min, centrifuged at 17,000 g for 5 min at 4°C, and supernatants were transferred to clean tubes, followed by evaporation to dryness under nitrogen.

Dried extracts were reconstituted in deionized water, and 5 μL was injected for analysis by ion chromatography (IC)-MS. IC mobile phase A (MPA; weak) was water, and mobile phase B (MPB; strong) was water containing 100 mM KOH. A Thermo Scientific Dionex ICS-5000+ system included a Thermo IonPac AS11 column (4 μm particle size, 250 x 2 mm) with column compartment kept at 30°C. The autosampler tray was chilled to 4°C.
The mobile phase flow rate was 360 µL/min, and the gradient elution program was: 0-5 min, 1% MPB; 5-25 min, 1-35% MPB; 25-39 min, 35-99% MPB; 39-49 min, 99% MPB; 49-50, 99-1% MPB. The total run time was 50 min. To assist the desolvation for better sensitivity, methanol was delivered by an external pump and combined with the eluent via a low dead volume mixing tee. Data were acquired using a Thermo Orbitrap Fusion Tribrid Mass Spectrometer under ESI negative ionization mode at a resolution of 240,000.

For HILIC analysis, samples were diluted in 90/10 acetonitrile/water containing 0.1% formic acid, then 10 µL was injected for analysis by liquid chromatography (LC)-MS. LC mobile phase A (MPA) was 95/5 (v/v) water/acetonitrile containing 20mM ammonium acetate and 20 mM ammonium hydroxide (pH–9), and mobile phase B (MPB) was acetonitrile. The LC system was a Thermo Vanquish with Xbridge BEH amide column (3.5 µm particle size, 100 x 4.6 mm; Waters Corp.) with column compartment kept at 30°C. The autosampler tray was chilled to 4°C. The mobile phase flow rate was 300 µL/min, and the gradient elution program was: 0-3 min, 85% MPB; 3-10 min, 85-30% MPB; 10-20 min, 30-2% MPB; 20-25 min, 2% MPB; 26-30 min, 2-85% MPB. The total run time was 30 min. Data were acquired using a Thermo Orbitrap Fusion Tribrid Mass Spectrometer under ESI positive and negative ionization mode at a resolution of 240,000.

Raw data files were imported to Thermo Trace Finder and Compound Discoverer software for spectrum database analysis. The relative abundance of each metabolite was normalized by total peak intensity.

Lipidomics
Ethanol, pre-cooled to -80°C, was added to murine fecal pellets at a ratio of 10 µL of absolute ethanol to 1 mg of fecal material. Samples were homogenized using a Precellys homogenizer, then 50 µL (equivalent to 5 mg of fecal material) of each homogenate was transferred to a Simport port pre-washed with methanol. To each sample 5 µL of Avanti SPLASH® LIPIDOMIX® Mass Spec Standard (330707) in methanol, 3 µL of 10 mM butylated hydroxytoluene in methanol, and 242 µL of -80 °C ethanol was added. The tubes were vortexed 5 min, then centrifuged at 4°C for 10 min at 17,000 g. The supernatants were then transferred to a Phenomenex Impact Protein Precipitation Plate (CE0-7565) and filtered through using a vacuum manifold. The pellets, resulting from centrifugation, were re-extracted with 300 µL of ethanol and the supernatants were once again passed through the protein precipitation plate. The plate wells were then rinsed with 200 µL of ethanol to elute residual lipids. The lipid extracts were transferred to new Simport tubes, pre-washed with methanol, and 200 µL of ethanol was used to wash the collection wells of the plate. The wash solutions were combined with the extracts and a centrifugal vacuum concentrator was used to dry the samples.

Ethanol containing 1% 10 mM butylated hydroxytoluene in methanol, and 2% Avanti SPLASH® LIPIDOMIX® Mass Spec Standard (330707) in methanol, pre-cooled to -80°C, was added to mouse serum at a ratio of 9 µL of absolute ethanol to 1 µL of serum. The tubes were vortexed 10 min, then centrifuged at 4°C for 15 min at 17,000 g. The supernatants were then collected for LCMS analysis.

Reverse Phase Liquid Chromatography
Dried samples from were reconstituted in 100 µL ethanol. The injection volume was 15 µL. The injection volume for serum was 10 μL. Mobile phase A (MPA) was 40:60 acetonitrile:0.1 % formic acid in 10 mM ammonium acetate. Mobile phase B (MPB) was 90:8:2 isopropanol:acetonitrile:0.1 % formic acid in 10 mM ammonium acetate. The chromatographic method included a Thermo Fisher Scientific Accucore C30 column (2.6 µm, 150 x 2.1 mm) maintained at 40 °C, autosampler tray chilling at 8 °C, a mobile phase flowrate of 0.200 mL/min, and a gradient elution program as follows: 0-7 min, 20-55% MPB; 7-8 min, 55-65% MPB; 8-12 min, 65% MPB; 12-30 min, 65-70% MPB; 30-31 min, 70-88% MPB; 31-51 min, 88-95% MPB; 51-53 min, 95-100% MPB; 53-60 min, 100% MPB; 60-60.1 min 100-20% MPB; 60.1-70 min, 20% MPB.

Mass Spectrometry
A Thermo Fisher Scientific Orbitrap Fusion Lumos Tribrid mass spectrometer with heated electrospray ionization source was operated in data dependent acquisition mode, in both positive and negative ionization modes, with scan ranges of 150 – 700 and 675 – 1500 m/z for the stool and 150 – 877 and 825– 1500 m/z for serum. An Orbitrap resolution of 120,000 (FWHM) was used for MS1 acquisition and a spray voltages of 3,600 and -2900 V were used for positive and negative ionization modes, respectively. Vaporizer and ion transfer tube temperatures were set at 275 and 300 °C, respectively. The sheath, auxiliary and sweep gas pressures were 35, 7, and 0 (arbitrary units), respectively. For MS2 and MS3 fragmentation a hybridized HCD/CID
approach was used. Each sample was analyzed using 4 x 10 µL injections making use of the two aforementioned scan ranges, in both ionization modes. Data were analyzed using Thermo Scientific LipidSearch software (version 4.2.23) and R scripts written in house.
Supplemental Figures for Yan et al.

A

C57BL/6 SPF

ABX cocktail:
Vancomycin
Neomycin
Ampicillin
Metronidazole (VNAM)

Flow cytometric analysis of hematopoietic populations

B

% Bone Marrow

LT-HSC MPP1 MPP2 MPP3 MPP4

C

LSK (% Bone Marrow)

D

LSK (% Bone Marrow)

E

LSK (Cells/bone)

F

SSC-A

Sca-1

Gr-1. B220. CD4. CD8. Ter199

CD45.1

CD45.2
Supplemental Figure 1. (A) General experimental design of antibiotic treatment. (B) Specific progenitor populations were also quantified from Cre-negative $Stat1^{fl/fl}$ mice. Bone marrow populations (Lin$^-$/Sca$^+$/cKit$^+$, LSK) were quantified from (C) Villin-Cre;$Stat1^{fl/fl}$ and (D) LepR-Cre;$Stat1^{fl/fl}$ after two weeks with or without VNAM by flow cytometry. (E) LSK populations were quantified from mice with or without STAT1 signaling in Vav-iCre;$Stat1^{fl/fl}$ mice after two weeks with or without VNAM by flow cytometry. (F) Gating strategies for flow cytometry of transplanted whole bone marrow. Results are (B, D, E) compiled from 2-4 independent experiments (B & E) ($n=6$-$10$ per group) or (D) ($n=5$-$8$) or (C) representative of 3 independent experiments ($n=3$). Graphs show mean ± SEM, with statistical significance determined by (B) Mann-Whitney test or (C-E) two-way ANOVA with Sidak’s multiple comparisons test. *$p<0.05$ **$p<0.01$ ****$p<0.0001$ n.s. not significant
Supplemental Figure 2. (A) Experimental schematic of administration of DAT to antibiotic treated mice. Bone marrow (B) granulocytes and (C) progenitors were quantified from mice treated with VNAM and oral gavage of DAT (20 mmol). Results are representative of (B & C) 2 independent experiments (n=3-4 per group). (B-C) Graphs show mean ± SEM, with statistical significance determined by one-way ANOVA with Tukey’s correction for multiple comparisons. *p<0.05 **p<0.01 ***p<0.001 n.s. not significant
**Supplemental Figure 3.** (A) Bone marrow granulocytes were measured from WT or Stat1−/− mice that received either 2 weeks of mock-treatment or VNAM as well as NOD1L (100 ug) or NOD2 agonist (300 ug) as control on the second week every 2-3 days. (B) 16s rRNA qPCR quantification from fecal pellets collected before and after 2 weeks of VNAM treatment or mock-treatment as well NOD1L. Fecal pellets were collected from a sampling of the mice from each group and pooled per group. The 16s rRNA gene from Blautia in a plasmid was used as the standard. Bone marrow granulocytes (C) percentage and (D) absolute numbers were quantified from control mice on two weeks of Kool-Aid water or NOD1L treated mice where NOD1L (100ug) was given every 2-3 days during the second week of Kool-Aid water. Results are representative of (A) 3 independent experiments (n= 4-6 per group), (B) 1 independent experiment (n= 3 technical replicates per group), or (C & D) 2 independent experiments (n= 4 per group). Graphs show mean ± SEM, with statistical significance determined by (A) two-way ANOVA with Sidak’s multiple comparisons test or (C & D) Student’s t-test. n.s. not significant.
Supplemental Figure 4. (A) The hematopoietic system of mice recover at different rates after stopping VNAM treatment. (B) Table of metabolites of interest from fecal metabolomics screen. Fold change of non-leukopenic mice relative to leukopenic mice. (C) Heatmaps of IC-MS, HILIC, and Lipidomics data from fecal metabolomics visualized using ClustVis. (D) Table of metabolites of interest from serum metabolomics screen. Fold change of non-leukopenic mice relative to leukopenic mice. (E) Heatmaps of IC-MS/HILIC and Lipidomics data from serum metabolomics visualized using ClustVis. Results are (A) representative of 3 independent experiments or (B-E) from 1 independent experiment.