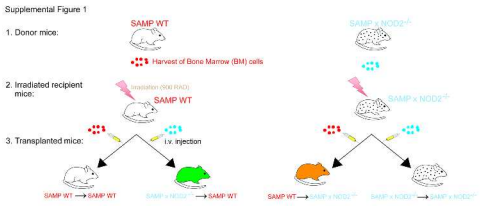
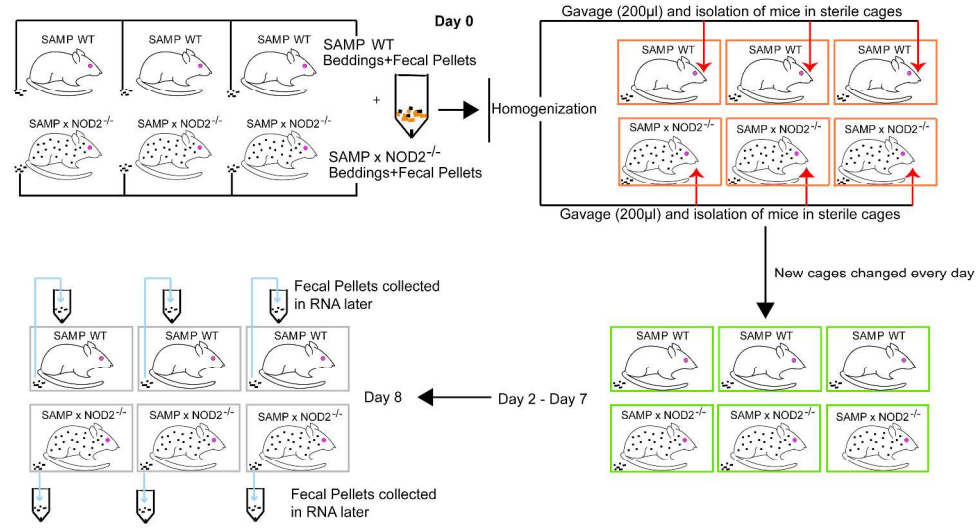


346x74mm (1000 x 1000 DPI)

Supplemental Figure 2



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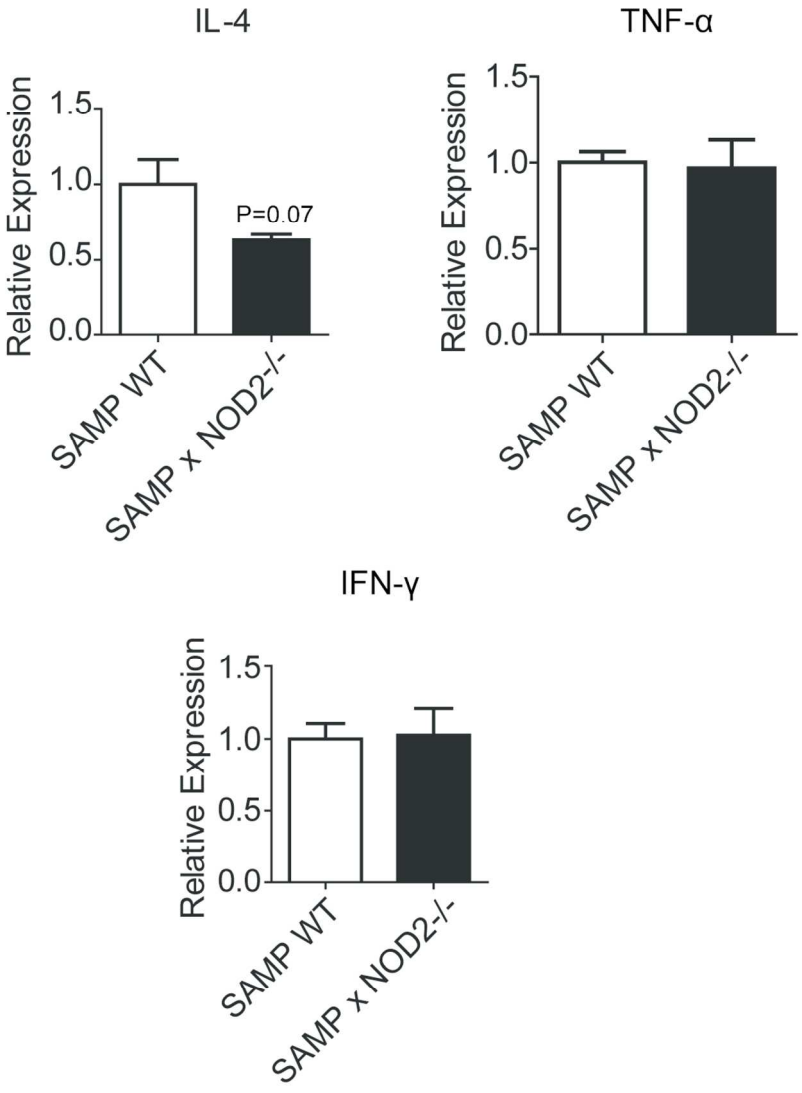
Supplemental Figure 3



79x103mm (400 x 400 DPI)

Supplementary Figure Legends

Figure S1. Related to figure 2. Generation of bone marrow chimera (BM) SAMP mice. Mice receiving BM transfer were irradiated (900 radiation absorbed dose) immediately before transplantation. BM was harvested from femurs and tibias of 10 wk old SAMP WT or SAMP NOD2$^{-/-}$ donor mice generating the following groups of chimeric mice: BM from SAMP NOD2$^{-/-}$ into irradiated SAMP WT mice (SAMP NOD2$^{-/-}$BM→ SAMP WT), and BM from SAMP WT mice into irradiated SAMP NOD2$^{-/-}$ mice (SAMP WT BM→ SAMP NOD2$^{-/-}$); irradiated SAMP WT transplanted with SAMP WT BM (SAMP WT BM→ SAMP WT) and irradiated SAMP NOD2$^{-/-}$ transplanted with SAMP NOD2$^{-/-}$ BM (SAMP NOD2$^{-/-}$→ SAMP NOD2$^{-/-}$) were used as controls.

Figure S2. Related to figure 5-7. Inter-subject pre-experimental fecal flora homogenization (IsPreFeH). Schematic representation of fecal homogenization protocol to artificially control for the confounding effects of gut flora in cage-to-cage divergence on spontaneous and inducible intestinal phenotypes. This approach is herein referred to as 'IsPreFeH', in which all investigational animal subjects are exposed to a composite of beddings and fecal material prior to experimentation. Single mice from diverse cages representing each the SAMP WT and KO$^{-/-}$ colonies in our research facility are exposed to a composite of fecal sample and pooled bedding material prior to the beginning of indicated experiments**.**

Figure S3. Related to figure 6. Gene Expression of cytokines in colonic tissue following DSS treatment. Gene expression of IL-4, TNFα and IFN-γ n colonic tissues of SAMP WT and SAMP NOD2$^{-/-}$ following treatment with 3% DSS for 1 week.

Supplemental Experimental Procedures

Stereomicroscopic 3D-pattern profiling of intestinal inflammation. Three dimensional-SM Assessment and Pattern Profiling (3D-SMAP*gut*) register form was used to capture qualitative and quantitative data and to produce a multidimensional scoring system that contains information regarding the extent of abnormal mucosa. The scale developed for the small intestine ranges from 0.000 to 1000.987. A score of 0 means healthy mucosa, while 652.895 indicates that the segment examined has an average of 65.2% of abnormal mucosa per linear (tubular) cm of gut examined, while decimals 895 indicate that the abnormality was due to lesion categories 8, 9 and 5 in order of prevalence. Here we focused on the presence of cobblestone formation (decimal category 8), which we discovered and documented for the first time is predictably present in 100% of SAMP mice. The finding that the NOD2 deletion resulted in a reduction of cobblestone ileitis is highly relevant because cobblestone lesions are characteristic and present in the majority of CD patients. In contrast, cobblestones are not present in C57BL/6J mice carrying a mutation on the TNF gene, TNFΔARE$^{-/+}$ mice, which results in over production of TNF and progressive spontaneous ileitis. The details, validity and cost-effective performance of our 3D-SMAP meyhodlogy compared to histology and scanning electron microscopy has been recently published (Rodriguez-Palacios et al., 2015a).

Inter-subject fecal homogenization (ISFecalH) protocol. The generation of mutant homozygous mice require the selection and separate maintenance of the ensuing line from the parental WT line. Because often KO colonies are bred separately over various generations from the parental line, with minimal flora exchange when raised under SPF or conventional conditions, we developed a fecal homogenization protocol to artificially and rapidly control for the confounding effect of gut flora cage-to-cage divergence on spontaneous and inducible intestinal phenotypes. The approach is herein referred as IsPreFeH (Rodriguez-Palacios et al., 2015a and 2015b) in which all investigational animal subjects are exposed to a composite of fecal material prior to experimentation as an alternative to cohousing which may not be always feasible with males and in all strains. In brief, single mice from diverse cages representing each the SAMP WT and SAMP NOD2$^{-/-}$ colonies in our research facility are exposed to a composite of fecal sample and pooled bedding material prior to the beginning of indicated experiments.

16S rRNA Encoding Gene Sequencing and Microbial Community Analyses. Community metagenomic DNA was extracted from fecal samples using the PowerSoil®-htp 96 Well Soil DNA Isolation Kit (MO BIO Laboratories, Inc.). Amplicons targeting the V4 region of 16S rRNA encoding genes from members of the Bacteria and Archaea using the 515F-806R primer set (Caporaso et al., 2011) were generated by PCR. Reactions (triplicate) for each sample used sterile DNase-free 96 well plates with appropriate (DNA template-free) negative controls using the 5 PRIME MasterMix (5 PRIME) and the following protocol: 95 ºC for 3 min, denaturation; and 35 cycles of 95 ºC for 30 s, 55 ºC for 45 s, then 72 ºC for 1.5 min, with an extension step at 72 ºC for 10 min (Caporaso et al., 2010) (Caporaso et al., 2010).

Triplicate PCR reactions were pooled together and the total amount of DNA quantified using the PicoGreen® dsDNA Assay (Life Technologies). Primer dimers were then removed from the pooled product using the UltraClean® PCR Clean-Up Kit (MoBio Laboratories, Inc.). The amount of amplicon in each pooled sample was normalized and sequenced on an Illumina MiSeq using 151 × 151 base pair paired-end sequencing at the Next Generation Sequencing Core at Argonne National Laboratory following procedures described by Caporaso *et al.* (Caporaso et al., 2010; Caporaso et al., 2011)

Raw sequence data was processed using the QIIME analysis pipeline and other bioinformatics toolkits (Edgar, 2010; McMurdie and Holmes, 2013). Joined reads were aligned to a database of reference sequences using PyNAST (Caporaso et al., 2011) and clustered into operational taxonomic units (OTUs) using UCLUST at 97% similarity. A consensus taxonomy from the Greengenes reference database was assigned to each sequence using the UCLUST consensus taxonomy assigner (Edgar, 2010; McMurdie and Holmes, 2013). Sequence data will be available in public software and repository MG-RAST (Meyer et al., 2008) http://metagenomics.anl.gov.

Metatranscriptome sequencing and analyses. Samples were homogenized and stored in RNAlater^{TM} (Ambion) at -80^{o}C. For subsequent RNA extraction, samples were thawed, centrifuged at 10,000g for 3min to pellet the sample, and RNAlater was removed. 500uL of HyPure^{TM} Molecular Biology Grade water (GE Healthcare LifeSciences) was then used to wash the sample of any residual RNAlater, centrifuged as before, and water was removed. Sample was then resuspended in fresh 500uL Hypure Molecular Biology Grade water and 300 uL of this slurry was used for RNA extraction while the remaining aliquot was frozen. RNA was extracted from the resuspended sample using the MOBIO PowerMicrobiome RNA extraction kit, with a final elution volume of 75 uL. RNA quality was assessed via

RNA integrity number (RIN) values obtained using the Agilent Bioanalyzer software (average RIN for the samples was 6.4; *n*=6). Metatranscriptome libraries were generated following standard protocols in the Argonne NGS core Laboratory (Hampton-Marcell et al., 2013).

Briefly, RNA was treated with Turbo DNA-free DNase (Life Technologies, Inc.), rRNA was removed utilizing the Ribo-Zero Bacteria Kit (Epicentre), and the remaining RNA checked using the Agilent Bioanalyzer to determine the extent of rRNA removal. Sequencing libraries were then generated with the mRNA using the ScriptSeq v2 RNA-Seq Library Kit (Epicentre) according to the manufacturer's instructions. Completed metatranscriptome libraries were then quality checked on the Agilent Bioanalyzer, quantified using Qubit (Invitrogen), diluted to 2nM and denatured for sequencing on the Illumina MiSeq. Libraries were sequenced two per run on a 151x151 Illumina MiSeq run. Storage and base analyses of the data were provided by the MG-RAST server at Argonne (Meyer et al., 2008). Reads were joined (mean length of 320 bp [SAMP-WT] and 324 bp [SAMP-NOD2 KO]), screened against the mouse genome (and matches removed), and artificial duplicate reads removed following screening using DRISEE (Keegan et al., 2012). The remaining sequences (mean sequence count of 945,942 [WT], and 967,392 [KO]) were then screened for protein and rRNA features and annotated using subsystems in MG-RAST. Additional off-line analyses were conducted on the annotated hits and abundances in R using the phyloseq, DESeq2, and edgeR packages (Robinson et al., 2010). Sequence data has MG-RAST project 12556 accession numbers 4618107.3-4618112.3).

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