Supplementary Methods

Cytospin and cell staining

Cells were sorted based on their surface marker expression, cytospun in PBS at 1000 rpm onto glass slides and stained with Hema 3 stain set (Fisher diagnostics) to identify cellular morphology.[1]

Ly6C^{hi} monocyte stimulation

Sorted Ly6C^{hi} monocytes from WT and *Nlrp6*$^{-/-}$ mice were cultured in complete RPMI with 10% FBS, penicillin/streptomycin and glutamine and stimulated with 20μM rosiglitazone (Cayman Chemicals). After 24-hour incubation at 37°C, supernatant was collected and cytokines measured by ELISA.

Gene Chip analysis

LP cells were isolated from WT or *Nlrp6*$^{-/-}$ mice at day 10 of AOM/2%DSS. Ly6C^{hi} monocytes were sorted on a FACS AriaIII (University of Michigan). RNA from sorted cells was extracted using the RNeasy Micro Kit (Qiagen), and contaminated DNA was removed using a DNA-free kit (Ambion). cDNA was prepared from 10 ng total RNA using the NuGen WT-Pico V2 kit (Ovation PicoSL WTA System V2 P/N 3312). Biotinylated single-stranded cDNA was then prepared from 3μg cDNA (Encore Biotin Module; catalog nos. 4200-12, 4200-60, and 4200-A01). Single-stranded cDNA was then fragmented, and 3.7 μg cDNA was hybridized for 20 h at 48 °C on Mouse Gene ST 2.1 strip arrays using the Affymetrix Gene Atlas System (software version 1.0.4.267). Arrays were scanned using the same system. Expression values were calculated using a robust multiarray average (RMA). RMA was calculated using the oligo package of bioconductor in R (Version 2.15.1; www.R-project.org). Ratios were generated using normalized raw expression values and then log2 transformed. Functional analyses of gene expression

changes were performed using Ingenuity Pathways Analysis (IPA) 8.0 (Ingenuity Systems; http://www.ingenuity.com). The analysis considered all genes from the data set that were associated with biologic functions in the Ingenuity Pathways Knowledge Base.[2] Heat map of hierarchical clustering of the genes from cytokine/chemokine pathways, based on IPA was generated. The microarray data are available in the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/gds) under the accession number GSE79631.

Supplementary Figure Legends

Supplementary Figure 1. NLRP6 is induced in lamina propria Ly6C^{hi} monocytes during DSS induced inflammation. WT and *Nlrp6*$^{-/-}$ mice were subjected to 3.5% DSS in drinking water for 5 days. On day 5 mice were sacrificed. NLRP6 expression was measured in **(a)** total LP cells and **(b)** CD3^{+}B220^{-}CD11b^{-} T cells, CD3^{-}B220^{+}CD11b^{-} B cells, CD3^{-}CD11b^{+}Ly6C^{hi}Ly6G^{-} monocytes and CD3^{-}CD11b^{+}Ly6C^{int}Ly6G^{+} neutrophils within the LP by qPCR. Data are presented as mean ± SEM; n=5/genotype; statistical analysis was completed using Mann-Whitney non-parametric U test, $p<0.05$.

Supplementary Figure 2. Ly6C^{hi} inflammatory monocytes are CCR2^{+}, which is required for protection against DSS-induced mortality in *Nlrp6*$^{-/-}$ mice. (a) Adoptive transfer of GFP^{+} Ly6C^{hi} monocytes at day 3.5 of 3.5% DSS results in infiltration of donor cells into the LP of recipient *Nlrp6*$^{-/-}$ mice. **(b)** Representative plots of Ly6C versus Ly6G staining of CD3^{-} CD11b^{+} LP cells (left) and percent CCR2^{+} cells within Ly6C^{hi} monocytes in the LP of DSS-treated WT and *Nlrp6*$^{-/-}$ mice (right). Staining for CCR2 in splenocytes isolated from *Ccr2*$^{-/-}$ mice as a negative control (far right). **(c)** Percent weight change of WT, *Nlrp6*$^{-/-}$, and *Nlrp6*$^{-/-}$ mice adoptively transferred with WT or *Ccr2*$^{-/-}$ Ly6C^{hi} monocytes with 3.5% DSS on days 0-7. **(d)** Kaplan–Meyer survival curves of mice treated with 7 days of 3.5% DSS. n=5 for WT and *Nlrp6*$^{-/-}$ + WT Ly6C^{hi} monocytes groups, n=8 for *Nlrp6*$^{-/-}$ and *Nlrp6*$^{-/-}$ + Ccr2$^{-/-}$ Ly6C^{hi} monocytes groups. **, $p<0.01$, between *Nlrp6*$^{-/-}$ and *Nlrp6*$^{-/-}$ + Ccr2$^{-/-}$ Ly6C^{hi} monocytes groups as compared to *Nlrp6*$^{-/-}$ + WT Ly6C^{hi} monocytes group.

Supplementary Figure 3. Adoptively transferred Ly6C^{hi} inflammatory monocytes infiltrate the lamina propria and are CX3CR1^{int}. (a) Age- and sex-matched *Nlrp6*$^{-/-}$ and

WT mice were treated 3.5% DSS for 5 days. On day 5, mice were sacrificed and composition of lamina propria cells analyzed. Representative plots of CX3CR1 expressing Ly6C^{hi} monocytes in DSS-treated WT and *Nlrp6*$^{-/-}$ mice in which 97% of LP-infiltrating inflammatory monocytes are CX3CR1^{+} in both WT and *Nlrp6*$^{-/-}$ mice. n=5 for each group of mice. **(b)** CX3CR1 staining of Ly6C^{hi} inflammatory monocytes sorted from the bone marrow of *CX3CR1*^{gfp} donor mice and adoptively transferred into *Ccr2*$^{-/-}$ recipient mice on day 3.5 of 3.5% DSS. **(c)** FACs analysis of LP cells isolated from DSS-treated *Ccr2*$^{-/-}$ recipient mice adoptively transferred with Ly6C^{hi} *CX3CR1*^{gfp} monocytes. CX3CR1-GFP expression in LP cells from DSS-treated *Ccr2*$^{-/-}$ recipient that were mock-transferred (left) or adoptively transferred with Ly6C^{hi} *CX3CR1*^{gfp} cells (middle). Histogram (right) showing CCR2 expression of CX3CR1-GFP^{-} and CX3CR1-GFP^{+} cells isolated from the designated *Ccr2*$^{-/-}$ recipient mice. CCR2^{+} cells are donor-derived.

Supplementary Figure 4. LP resident macrophages do not exhibit impaired TNFα production. Resident LP macrophages (CD11b^{+}Ly6C$^{low/-}$F4/80^{high}Ly6G^{-}) were sorted from the LP of WT or *Nlrp6*$^{-/-}$ mice at day 10 of AOM/DSS as cells and gene induction was measured by qPCR. Data are representative of three independent experiments, mean ± SEM; n=14, for both WT and *Nlrp6*$^{-/-}$ mice. * - $p<0.05$, as compared to WT.

Supplementary Figure 5. NLRP6$^{-/-}$ Ly6C^{hi} monocytes have impaired TNFα production in response to DSS-induced epithelial injury. (a) Induction of TNFα in T cells, Ly6C^{hi} monocytes and total LP isolated from WT and *Nlrp6*$^{-/-}$ mice after 5 days of 3.5% DSS as measured by qPCR. (b) Ly6C^{hi} monocytes from WT or *Nlrp6*$^{-/-}$ mice were sorted from the LP after 5 days of 3.5% DSS and production of TNFα and IL1β was measured by ELISA. **(c)** Fecal lipocalin-2 levels were measured by ELISA before and

after 5 days of 3.5% DSS. Data are presented as mean ± SEM; n=5/genotype; statistical analysis was completed using Mann-Whitney non-parametric U test, $p<0.05$.

Supplementary Figure 6. Rosiglitazone, a PPARγ agonist, induces NLRP6 expression and upregulates TNFα production in Ly6C^{hi} inflammatory monocytes. Ly6C^{hi} monocytes were sorted from BM of WT or *Nlrp6*$^{-/-}$ mice and stimulated with 20 μM rosiglitazone in complete RPMI. After 24-hour incubation, RNA was isolated from cells and mRNA levels of Nlrp6 **(a)** and TNFα **(b)** were measured by qPCR. Data are representative of two independent experiments, mean ± SEM; n=8, for both WT and Nlrp6$^{-/-}$ mice. * - $p<0.05$, as compared to *Nlrp6*$^{-/-}$ (A) or as compared to WT (B).

Supplementary Figure 7. Recruitment of Ly6C^{hi} monocytes into the colon lamina propria of *Nlrp6*$^{-/-}$ mice is not impaired during the acute inflammatory response to DSS. Age- and sex-matched *Nlrp6*$^{-/-}$ and WT mice were subjected to AOM/DSS treatment. Lamina propria cells were isolated and stained on the indicated days. **(a)** Representative plots of sorted cells populations. **(b)** Percent frequency of Ly6C^{hi} monocytes in total LP cells (left) and absolute number of Ly6C^{hi} monocytes (right) in the colon LP during AOM/DSS treatment. Kinetics for days 0, 10, 16 are shown. Data are representative of five independent experiments, mean ± SEM; n=17, n=18, for WT and *Nlrp6*$^{-/-}$ mice respectively (day 0), n=19 for both WT and *Nlrp6*$^{-/-}$ mice (day 10) and n=11, for both WT and *Nlrp6*$^{-/-}$ mice (day 16). *, ** - $p<0.05$, $p<0.001$, respectively, as compared to day 0 time point of both genotypes.

Supplementary Figure 8. Differential expression of multiple cytokines and chemokines between WT and *Nlrp6*$^{-/-}$ Ly6C^{hi} monocytes in response to AOM/DSS

treatment. Ly6C^{hi} monocytes were sorted from the lamina propria of WT and *Nlrp6*$^{-/-}$ mice on day 10 of AOM/DSS. RNA was extracted and hybridized to the mouse 2.1 ST array. Ratios were generated using normalized raw expression values and then log2 transformed. Functional analyses of gene expression changes were performed using Ingenuity Pathways Analysis (IPA). Heat map of hierarchical clustering of the genes from cytokine/chemokine pathways based on IPA are shown. Gene chip data are from cells sorted from 4 mice/genotype.

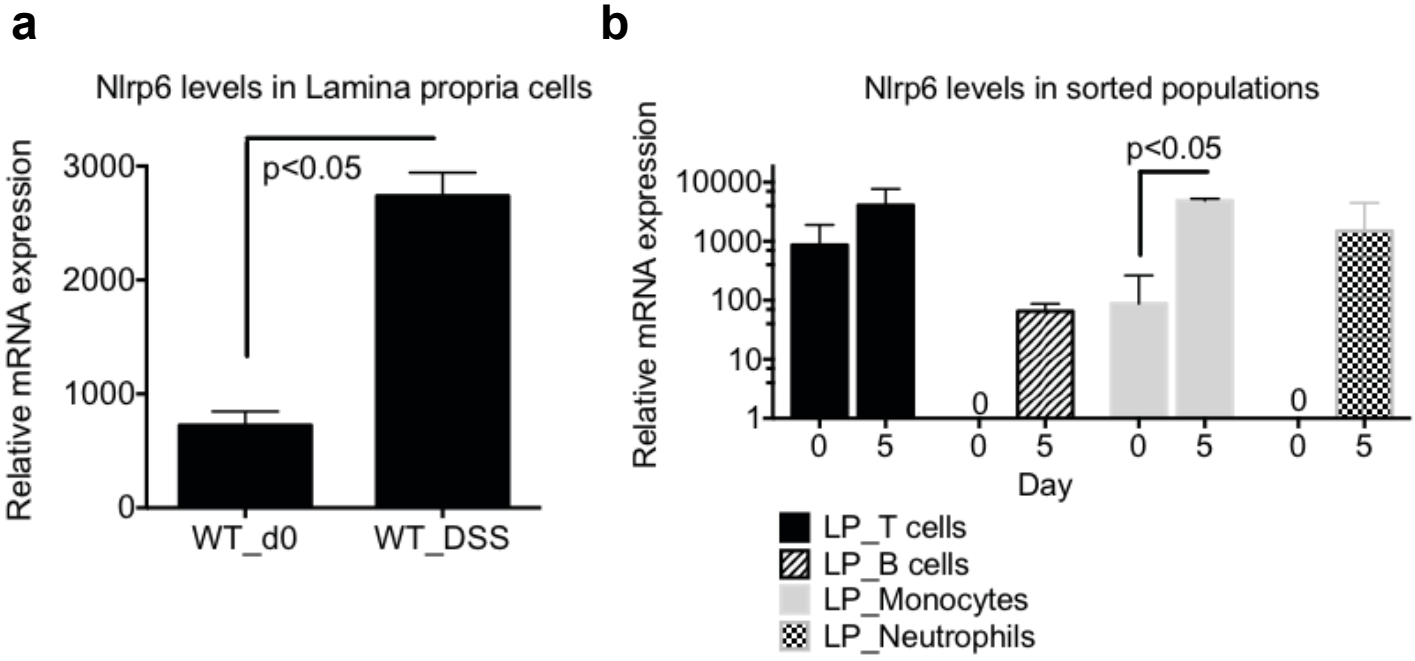
Supplementary Figure 9. NLRP6 deficient Ly6C^{hi} monocytes have reduced IL18 production during the acute inflammatory response to DSS. Ly6C^{hi} monocytes from WT or *Nlrp6*$^{-/-}$ mice were sorted from the LP after 5 days of 3.5% DSS, and IL-18 levels were measured by ELISA. Data are presented as mean ± SEM; n=5/genotype.

Supplementary Table I. Histological Scoring System

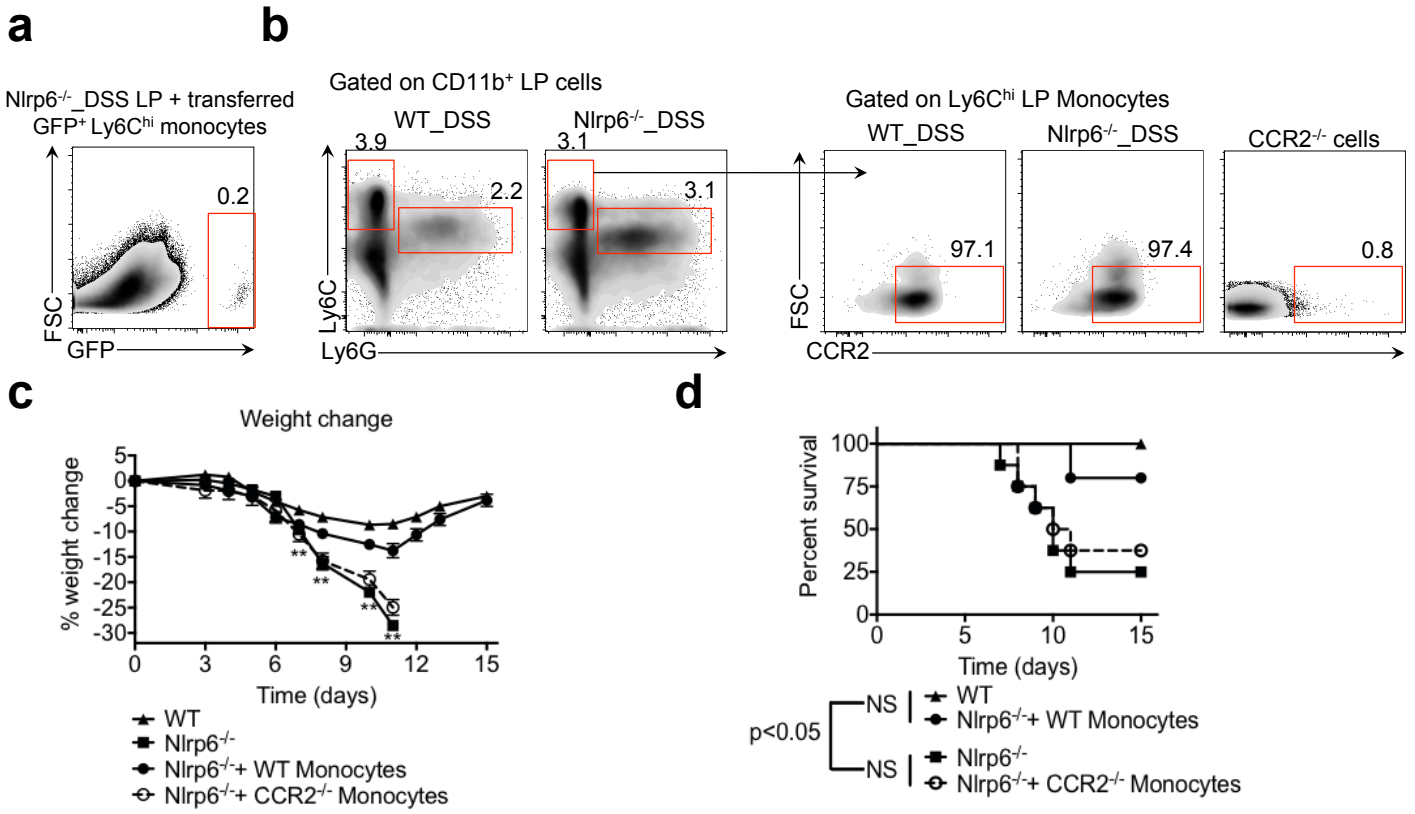
References

1. Zhan Y, Chen PJ, Sadler WD, Wang F, Poe S, Nunez G et al. Gut microbiota protects against gastrointestinal tumorigenesis caused by epithelial injury. Cancer Res 2013; 73(24): 7199-7210.

2. Troiani T, Martinelli E, Napolitano S, Vitagliano D, Ciuffreda LP, Costantino S et al. Increased TGF-alpha as a mechanism of acquired resistance to the anti-EGFR inhibitor cetuximab through EGFR-MET interaction and activation of MET signaling in colon cancer cells. Clin Cancer Res 2013; 19(24): 6751-6765.

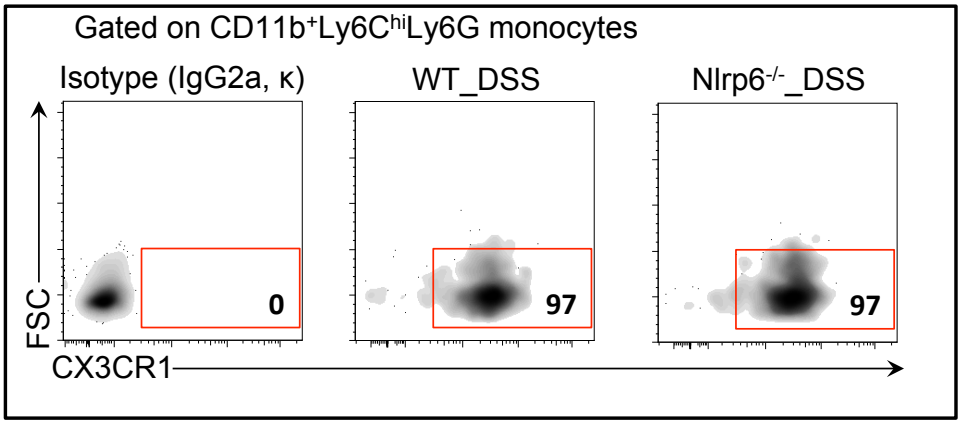


Supplementary Figure 1

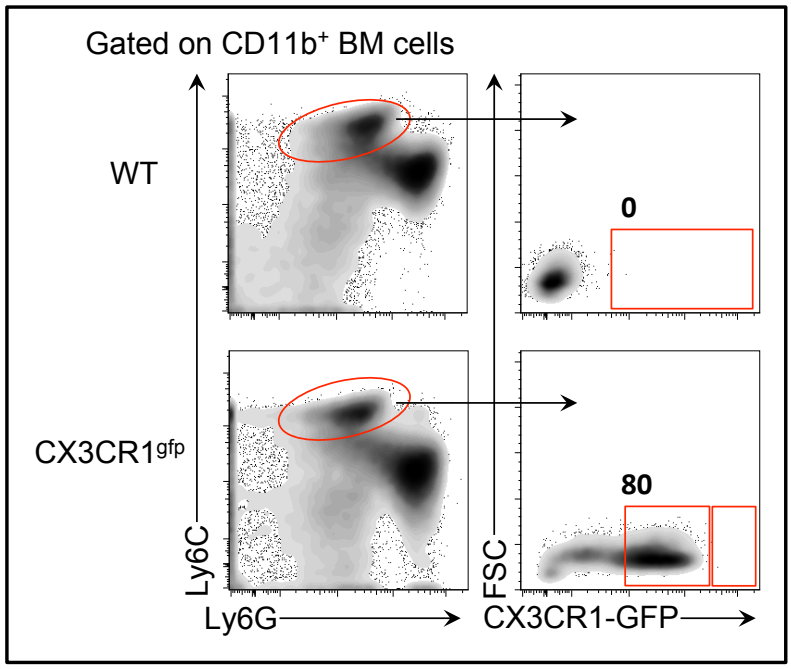


Supplementary Figure 2

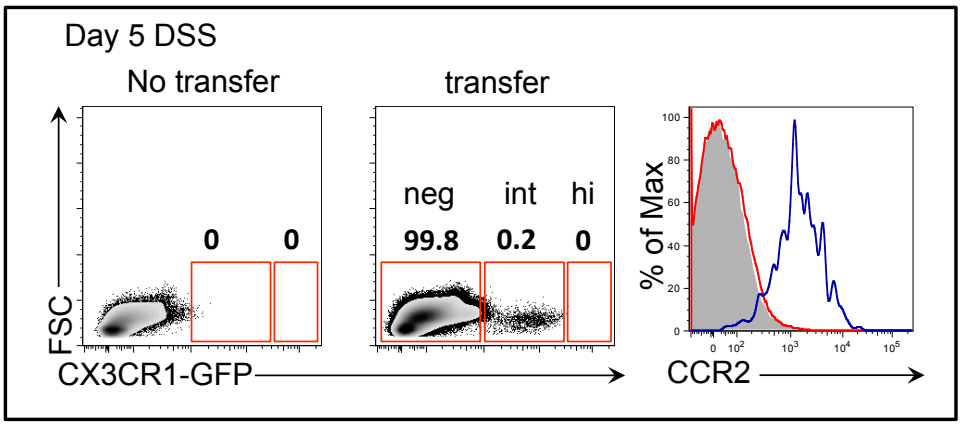
a **Lamina propria**



b **Bone marrow**

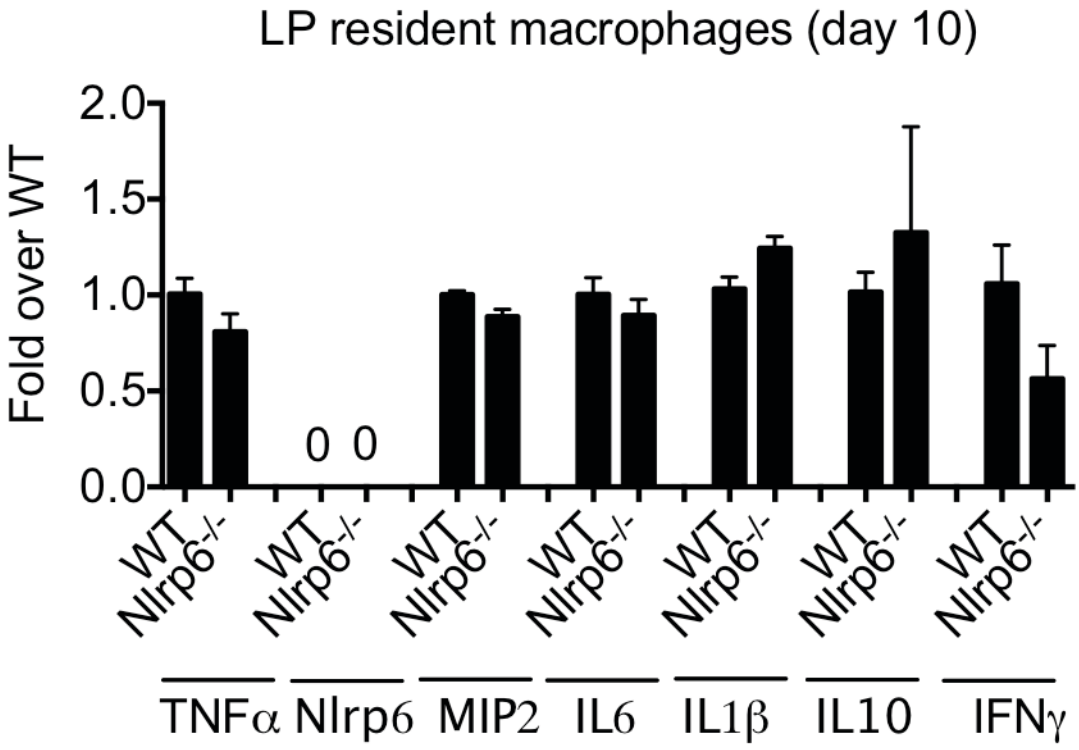


c **Lamina propria**

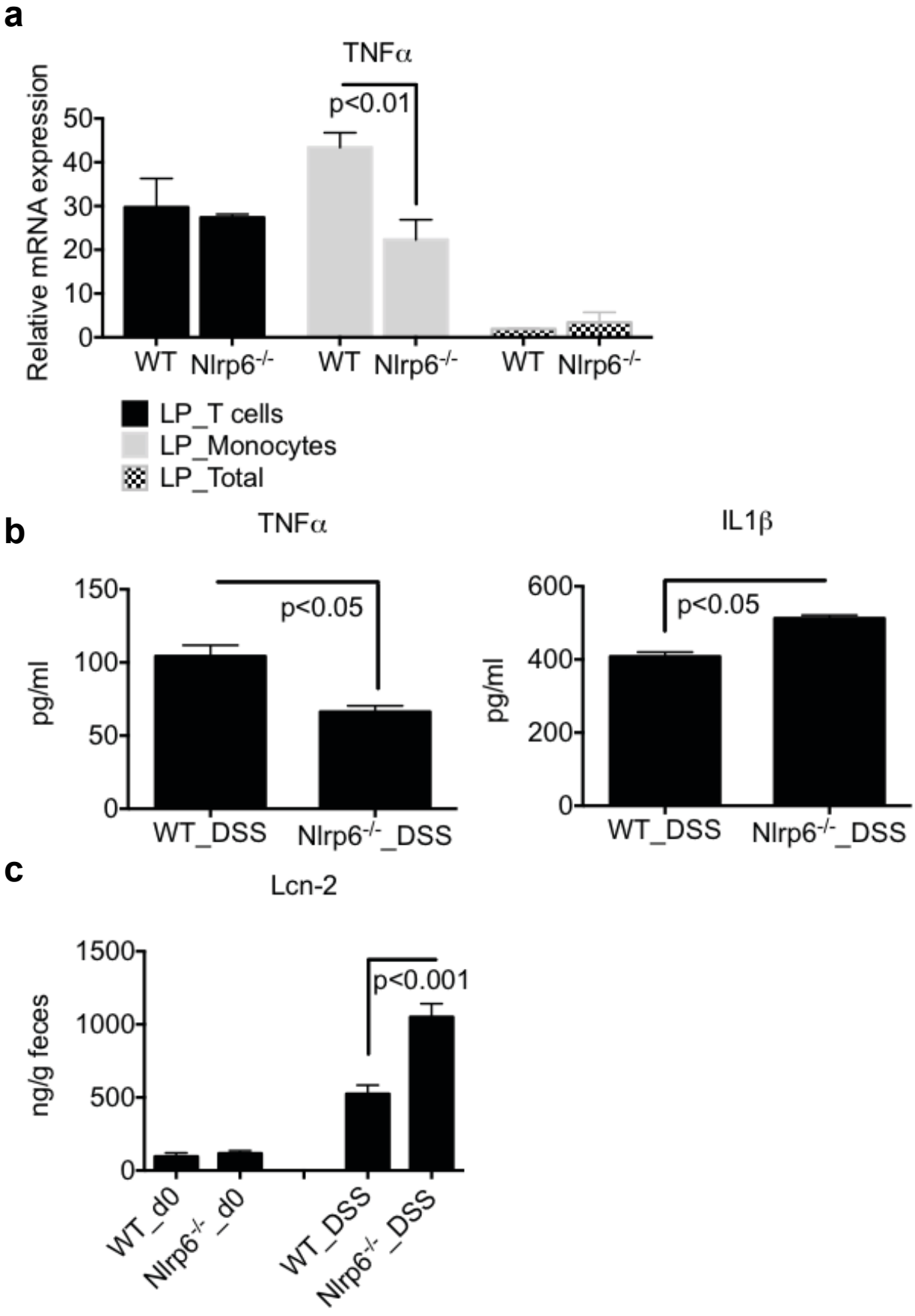


LP cells from *Ccr2*$^{-/-}$ mice mock transferred
LP cells from *Ccr2*$^{-/-}$ mice transferred with Ly6C^{hi} donor cells (gated on CX3CR1-GFP^{-})
LP cells from *Ccr2*$^{-/-}$ mice transferred with Ly6C^{hi} donor cells (gated on CX3CR1-GFP^{+})

Supplementary Figure 3



Supplementary Figure 4



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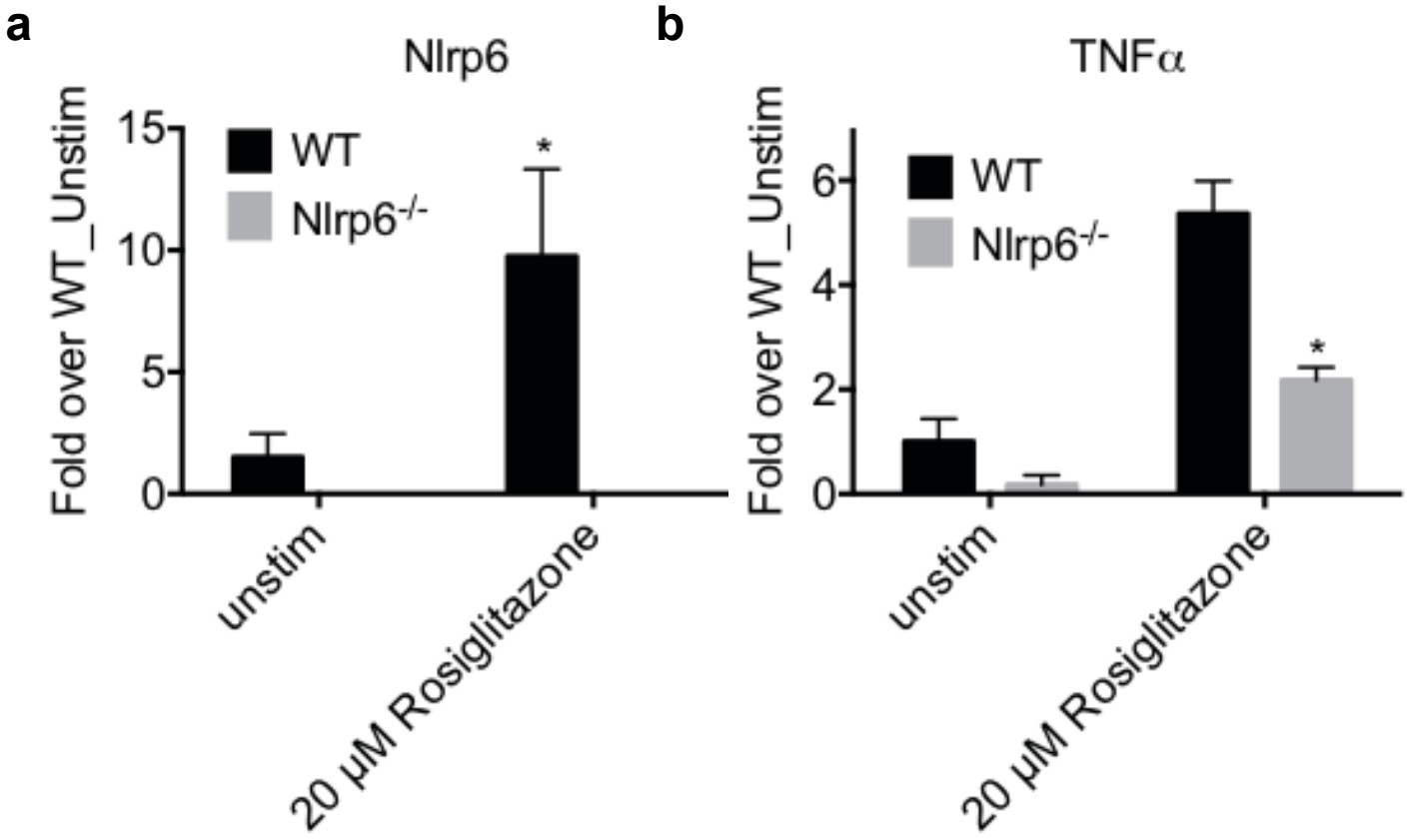
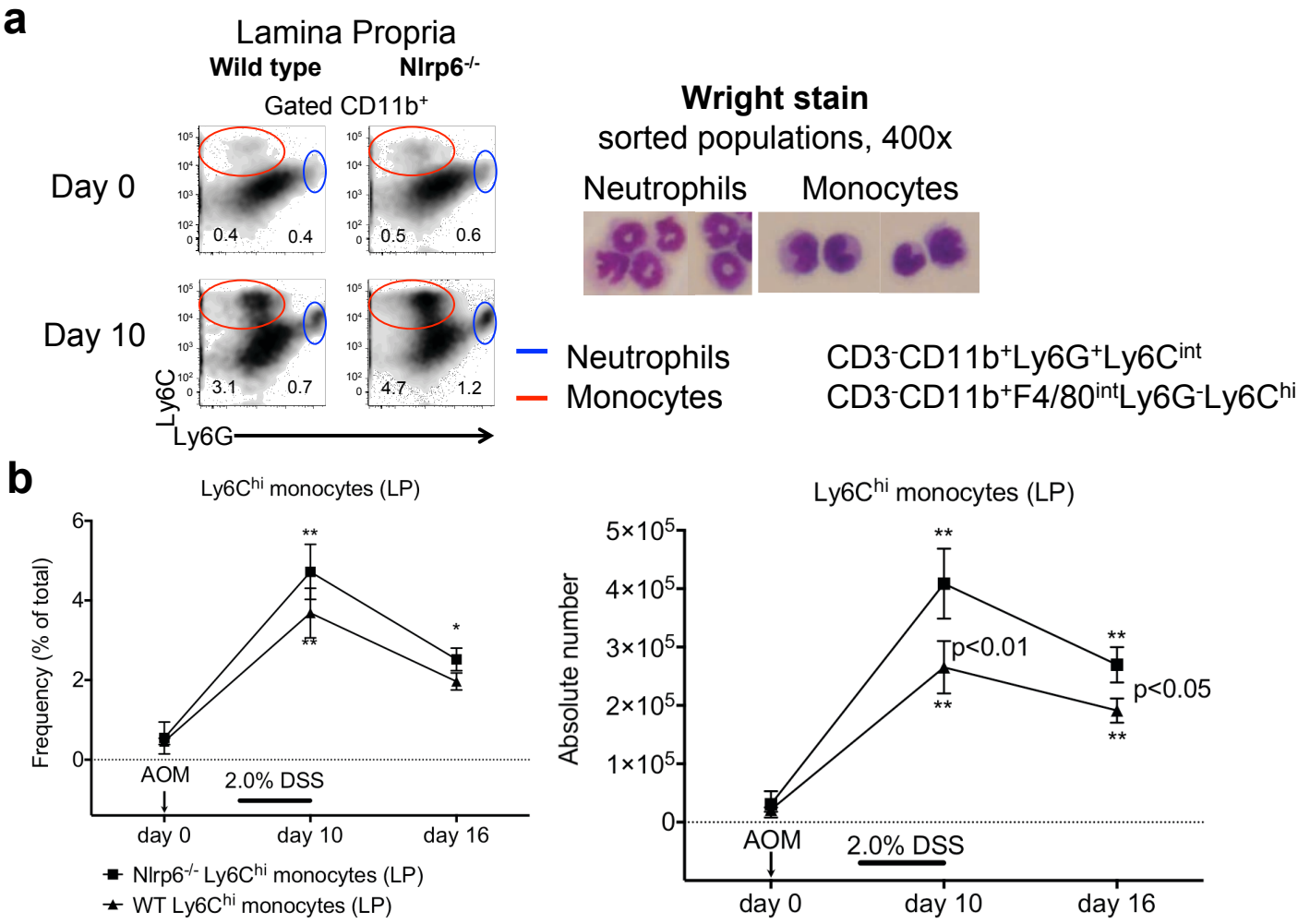
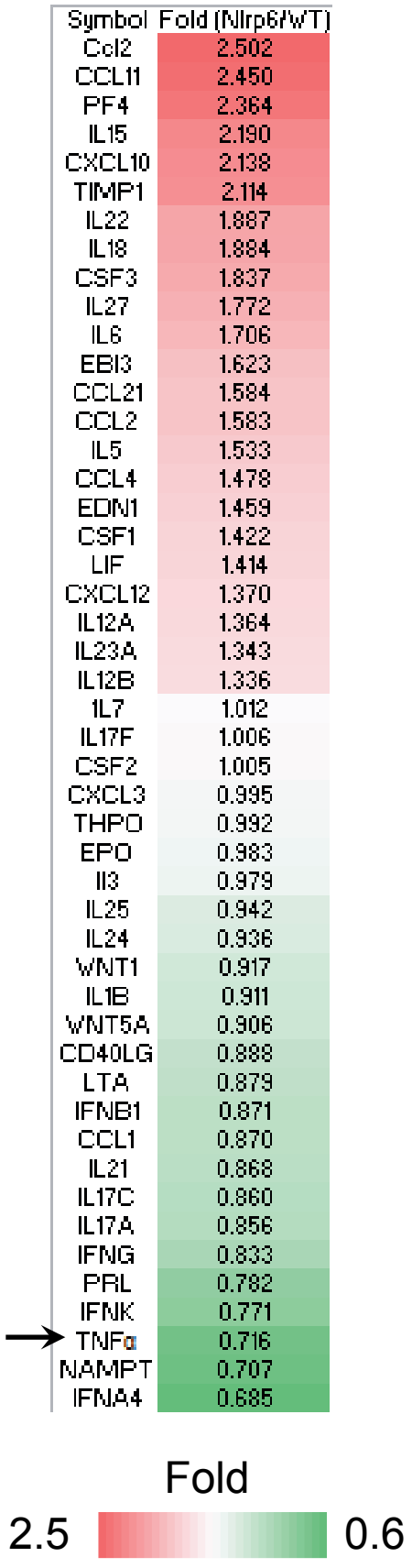


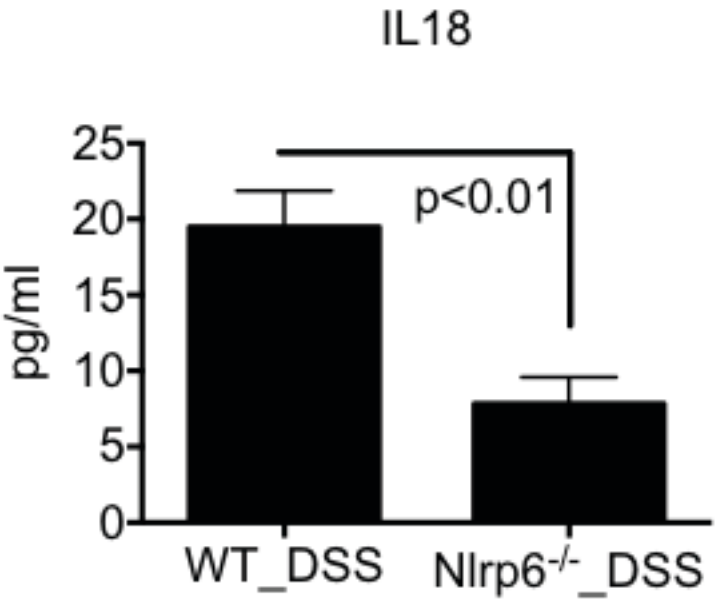
Supplementary Figure 6



Supplementary Figure 7



Supplementary Figure 8



Supplementary Figure 9

Supplementary Table I: Histological Scoring System

Lesion	Score =0	Score = 1	Score =2	Score =3	Score =4
Inflammation	No inflammation	mild infiltration of neutrophils confined to the amina propria	moderate infiltration of neutrophils or mixed inflammatory cells in the lamina propria and submucosa	Severe, mixed inflammation extend through the lamina propria and submucosa	Severe, transmural inflammatory infiiltrate
Epithelial damage	Within normal limits	Occasional dilated glands and/or single cell necrosis within glands	Widespread gland loss	Surface epithelial erosion with loss of glands and collapse of lamina propria	Ulceration
Hyperplasia	No hyperplasia	Minimal proliferation	Prominent proliferation, focally severe or throughout the field	Dysplasia	Adenoma