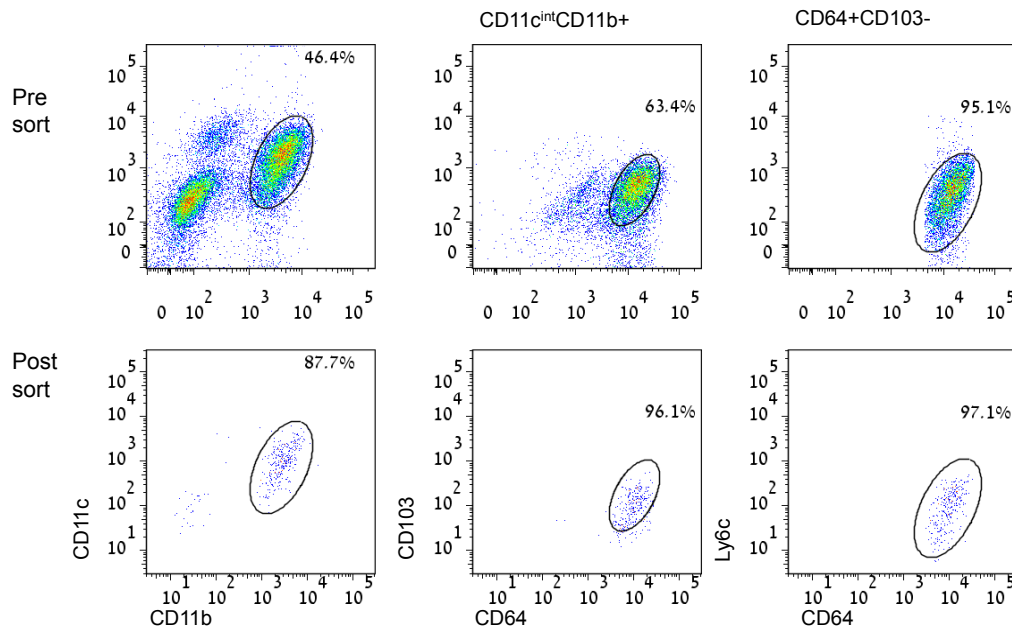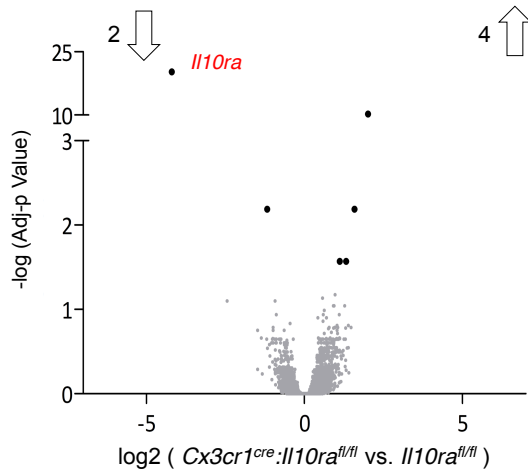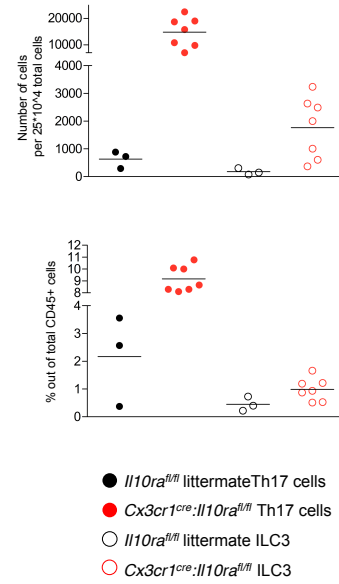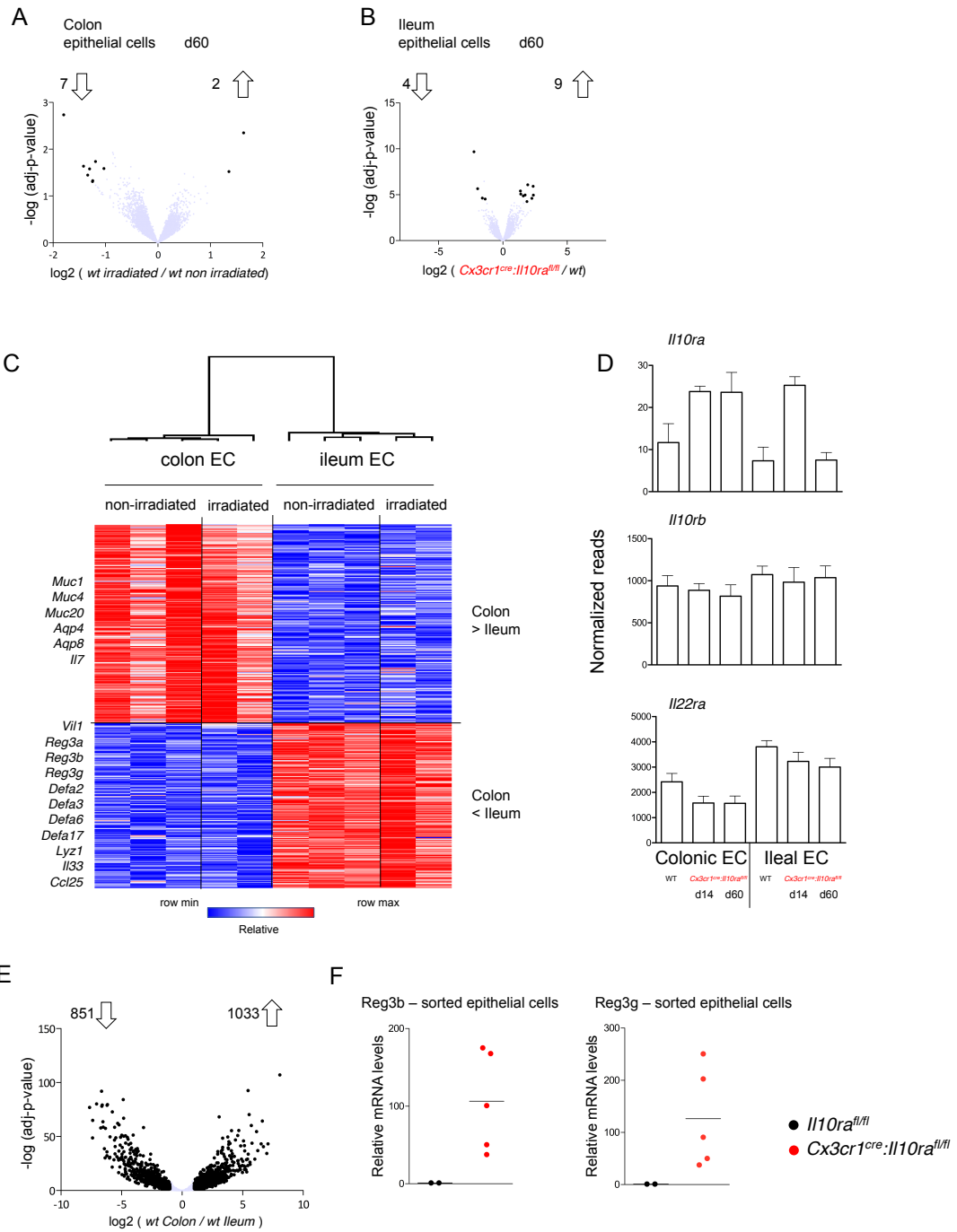Supp Figure 1

Supplementary Figure 1. Gating strategies and transcriptome analysis of animals exposed to antibiotics

This figure includes data related to main text Figures 1 and 3

(A) Gating strategy for sorting colonic lamina propria macrophages

(B) Volcano plot of statistical significance (-log10 p-value) against log 2 ratio of macrophages sorted from the colonic lamina propria of $Cx3cr1^{cre}:Il10ra^{fl/fl}$ and $Il10ra^{fl/fl}$ co-housed littermates continuously treated with broad spectrum antibiotics given upon weaning, based on RNA-seq data. Significantly up or down regulated genes (fold change>2, adj-p value<0.05) are in black. Cre transgene positive mice were collected from 2 independent experiments (n=2 in each), littermates are from one experiment (n=3).

(C) Quantification of flow cytometry analysis presented in Fig1F.

A Colon epithelial cells   d60

7 ⬇   2 ⬆

-log (adj-p-value) vs log2 (*wt irradiated / wt non irradiated*)

B Ileum epithelial cells   d60

4 ⬇   9 ⬆

-log (adj-p-value) vs log2 (*Cx3cr1^{cre}:Il10ra^{fl/fl} / wt*)

C

colon EC   ileum EC

non-irradiated irradiated non-irradiated irradiated

Muc1
Muc4
Muc20
Aqp4
Aqp8
Il7

Colon > Ileum

Vil1
Reg3a
Reg3b
Reg3g
Defa2
Defa3
Defa6
Defa17
Lyz1
Il33
Ccl25

Colon < Ileum

row min   row max
Relative

D

Il10ra

Normalized reads

Il10rb

Il22ra

Colonic EC   Ileal EC

WT Cx3cr1^{cre}:Il10ra^{fl/fl} WT Cx3cr1^{cre}:Il10ra^{fl/fl}
    d14 d60   d14 d60

E

851 ⬇   1033 ⬆

-log (adj-p-value) vs log2 (*wt Colon / wt Ileum*)

F

Reg3b – sorted epithelial cells

Relative mRNA levels

Reg3g – sorted epithelial cells

Relative mRNA levels

● *Il10ra^{fl/fl}*
● *Cx3cr1^{cre}:Il10ra^{fl/fl}*

Supp Figure 2

3

Supplementary Figure 2. Transcriptome analysis of epithelial cells of *Villin^cre^:R26-tdTomato* mice.

This figure includes data related to main text Figure 2

(A-B) Volcano plot of statistical significance (-log10 p-value) against log 2 ratio based on RNA-seq data between A - colonic epithelial cells sorted from [*Cx3cr1^gfp/+^ > Villin^cre^:r26-tdTomato*] BM chimeras and *Villin^cre^:r26-tdTomato* mice or B – ileal epithelial cells sorted from [*Cx3cr1^cre^Il10ra^fl/lf^ >Villin^cre^:r26-tdTomato*] BM chimeras *and Villin^cre^:r26-tdTomato* mice. Significantly up or down regulated genes (fold change>2, adj-p value<0.05) are in black.

(C) Heat map displaying RNA-seq data of sorted colonic and ileal epithelial cells from [*Cx3cr1^gfp/+^ > Villin^cre^:r26-tdTomato*] BM chimeras and *Villin^cre^:r26-tdTomato* mice**.** Presented are 1884 genes significantly differentially expressed (fold change>2, adj-p value<0.05) between colon and ileum. Normalized read number were log transformed and clustered by a Pearson correlation test and number of partition clusters was set to two**.** Samples were hierarchically clustered by a Pearson correlation test.

(D) RNA-seq normalized read numbers of indicated genes expressed by colonic and ileal epithelial cells extracted from *Villin^cre^:r26-tdTomato* mice and [*Cx3cr1^cre^Il10ra^fl/lf^ >Villin^cre^:r26-tdTomato*] BM chimeras at indicated time points.

(E) Volcano plot of statistical significance (-log10 p-value) against log 2 ratio between colonic and ileal epithelial cells sorted from *Villin^cre^:r26-tdTomato* mice based on RNA-seq data. Significantly up or down regulated genes (fold change>2, adj-p value<0.05) are in black.

(F) qRT-PCR analysis of *reg3b* and *reg3g* expression in sorted colonic epithelial cells (CD45-fraction) of *Cx3cr1^cre^:Il10ra^fl/fl^* mice and littermate controls

A

● *Cx3cr1$^{gfp+/-}$*
● *Cx3cr1^{cre}:Il10ra$^{fl/fl}$*
○ *Cx3cr1^{cre}:Il10ra$^{fl/fl}$:Il23a$^{fl/fl}$*

B

Il22

Reg3b

Reg3g
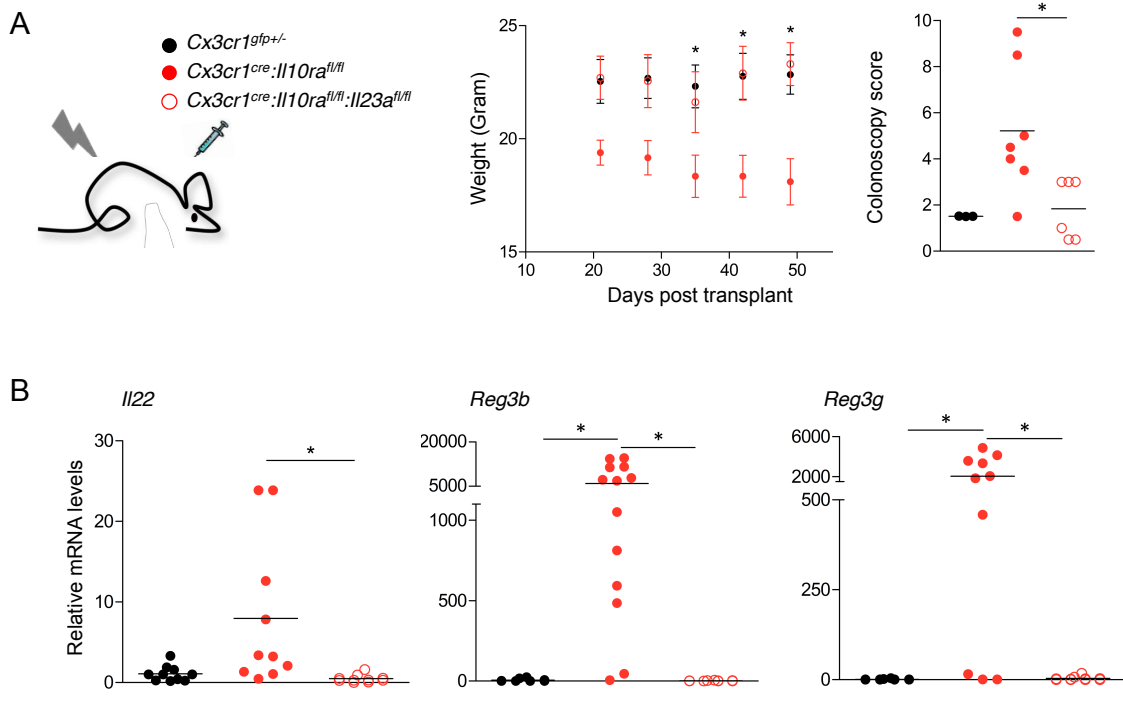
Supplementary Figure 3. Lack of neutrophil infiltrates in the *lamina propria* of *Cx3cr1^{cre}:Il10ra$^{fl/fl}$:Il23a$^{fl/fl}$* mice.

This figure includes data related to main text Figure 6
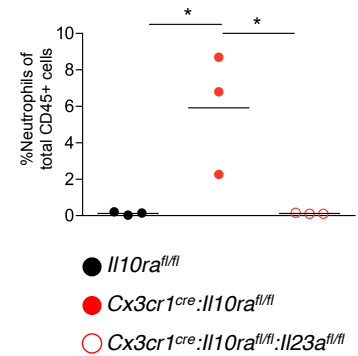
(A) Representative plots of flow cytometry analysis of the colonic *lamina propria* of indicated mouse strains.

(B) Quantification of flow cytometry analysis according to gating strategy indicated in A. data are from one experiment.
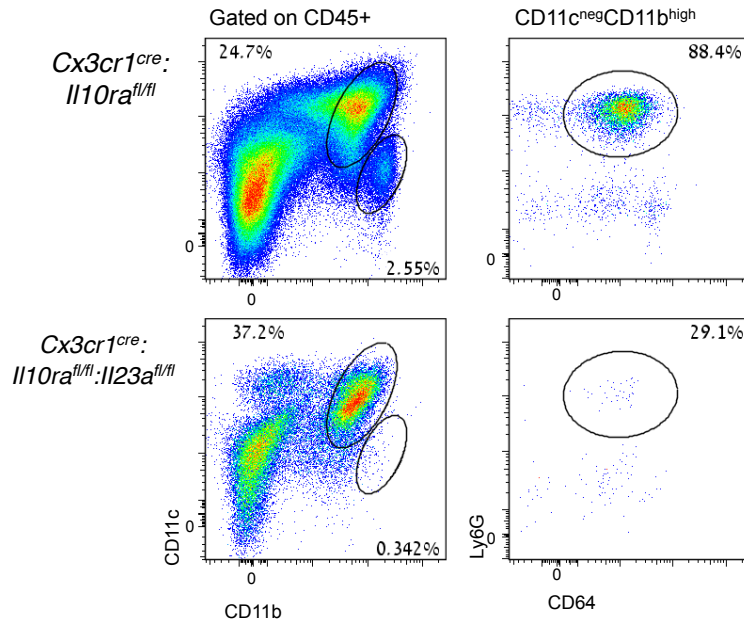
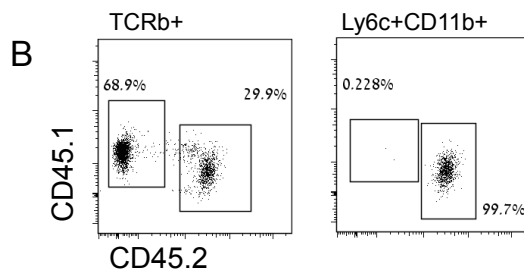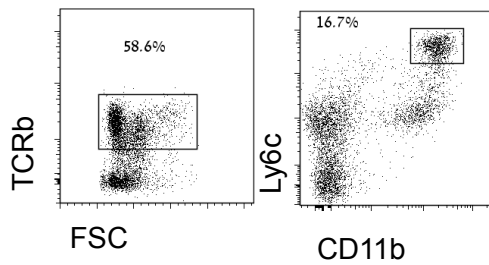**Supplementary Figure 4. Macrophage-derived Il23a is critical for colitis induction in BM chimeras model.**

This figure includes data related to main text Figure 3

(A) Weight and colonoscopic analysis of [*Cx3cr1^cre^:Il10ra^fl/fl^* > WT], [*Cx3cr1^gfp/+^* > WT] or [*Cx3cr1^cre^:Il10ra^fl/fl^:Il23a^fl/fl^* > WT] BM chimeras. Colonoscopy was performed 6 weeks post-transplant.
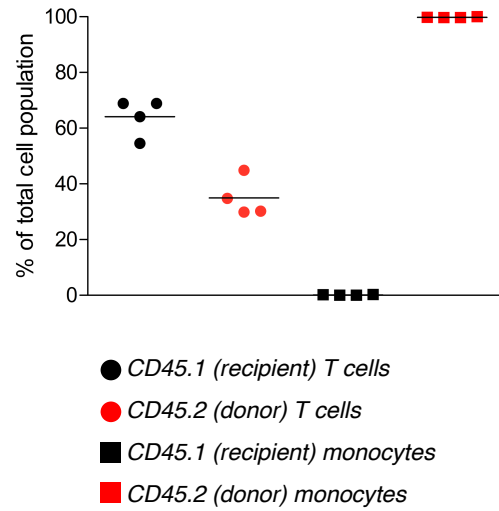
(B) qRT-PCR analysis of *il22, reg3b* and *reg3g* expression in colonic whole tissue RNA extracts of indicated BM chimeric mice.

Data are collected from 2 independent experiments, n>=3 in each.

A [CD45.2->CD45.1] BM chimeras – blood analysis

TCRb / FSC — 58.6%

Ly6c / CD11b — 16.7%

B

TCRb+ — CD45.1 / CD45.2 — 68.9%, 29.9%

Ly6c+CD11b+ — 0.228%, 99.7%

C

% of total cell population

● CD45.1 (recipient) T cells
● CD45.2 (donor) T cells
■ CD45.1 (recipient) monocytes
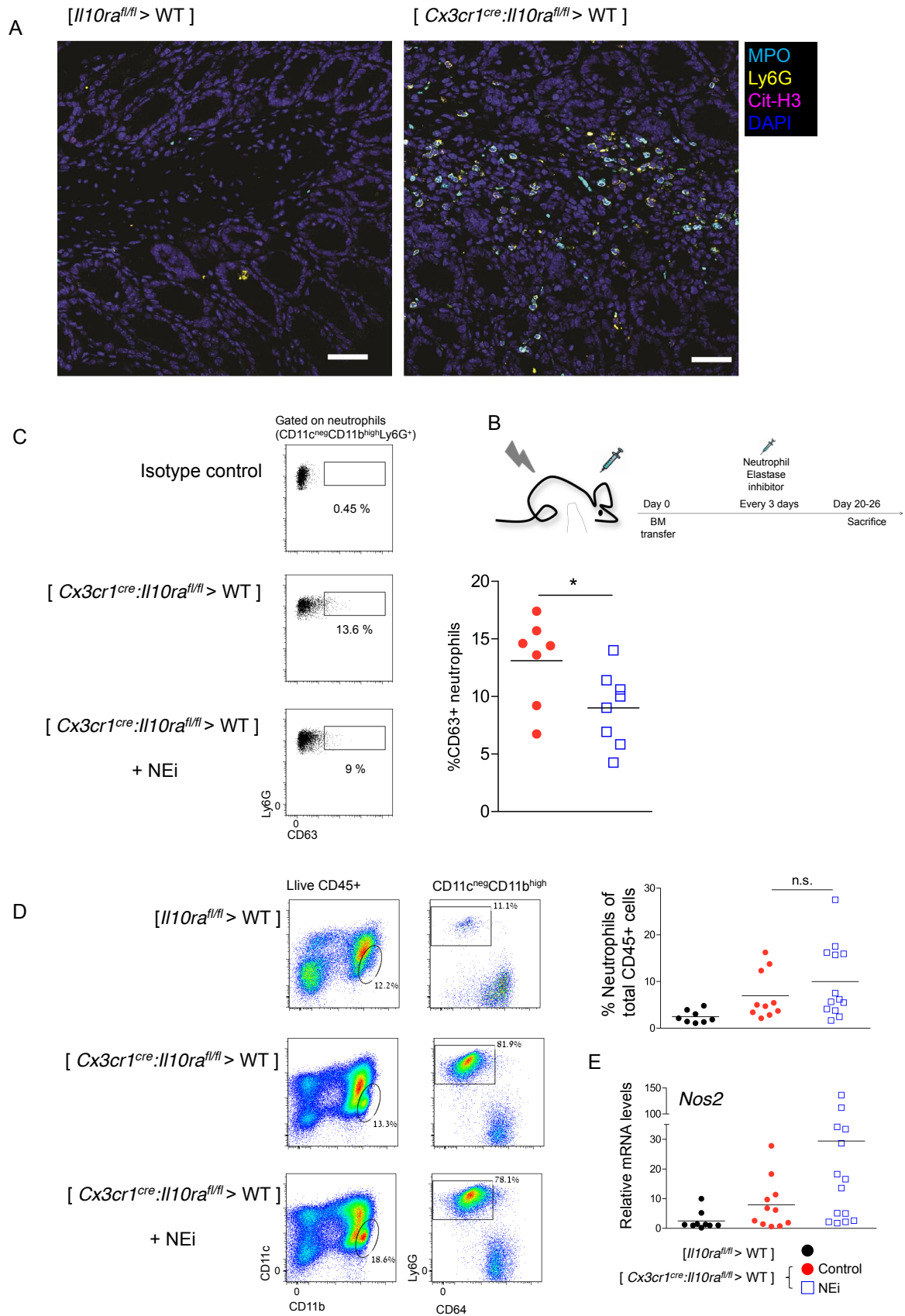■ CD45.2 (donor) monocytes

Supplementary Figure 5. Chimerism of blood monocytes and T cells.

(A-B) Representative flow cytometry analysis of blood withdrawn from [CD45.2>CD45.1] BM chimeras one month post transfer.

(C) Quantification of flow cytometry analysis presented in A-B.

A

[*Il10ra^fl/fl* > WT]   [*Cx3cr1^cre:Il10ra^fl/fl* > WT]

MPO
Ly6G
Cit-H3
DAPI

C

Gated on neutrophils
(CD11c^neg CD11b^high Ly6G^+)

Isotype control   0.45 %

[*Cx3cr1^cre:Il10ra^fl/fl* > WT]   13.6 %

[*Cx3cr1^cre:Il10ra^fl/fl* > WT]
+ NEi   9 %

Ly6G
CD63

B

Neutrophil
Elastase
inhibitor

Day 0   Every 3 days   Day 20-26
BM
transfer   Sacrifice

%CD63+ neutrophils

20
15
10
5
0

*

D

Llive CD45+   CD11c^neg CD11b^high

[*Il10ra^fl/fl* > WT]   12.2%   11.1%

[*Cx3cr1^cre:Il10ra^fl/fl* > WT]   13.3%   81.9%

[*Cx3cr1^cre:Il10ra^fl/fl* > WT]
+ NEi   18.6%   78.1%

CD11c
CD11b   Ly6G
CD64

% Neutrophils of
total CD45+ cells

30
20
10
0

n.s.

E

Nos2

Relative mRNA levels

150
100
30
10
0

[*Il10ra^fl/fl* > WT]   ●

[*Cx3cr1^cre:Il10ra^fl/fl* > WT] [● Control
                                    □ NEi

Supp Figure 6

8

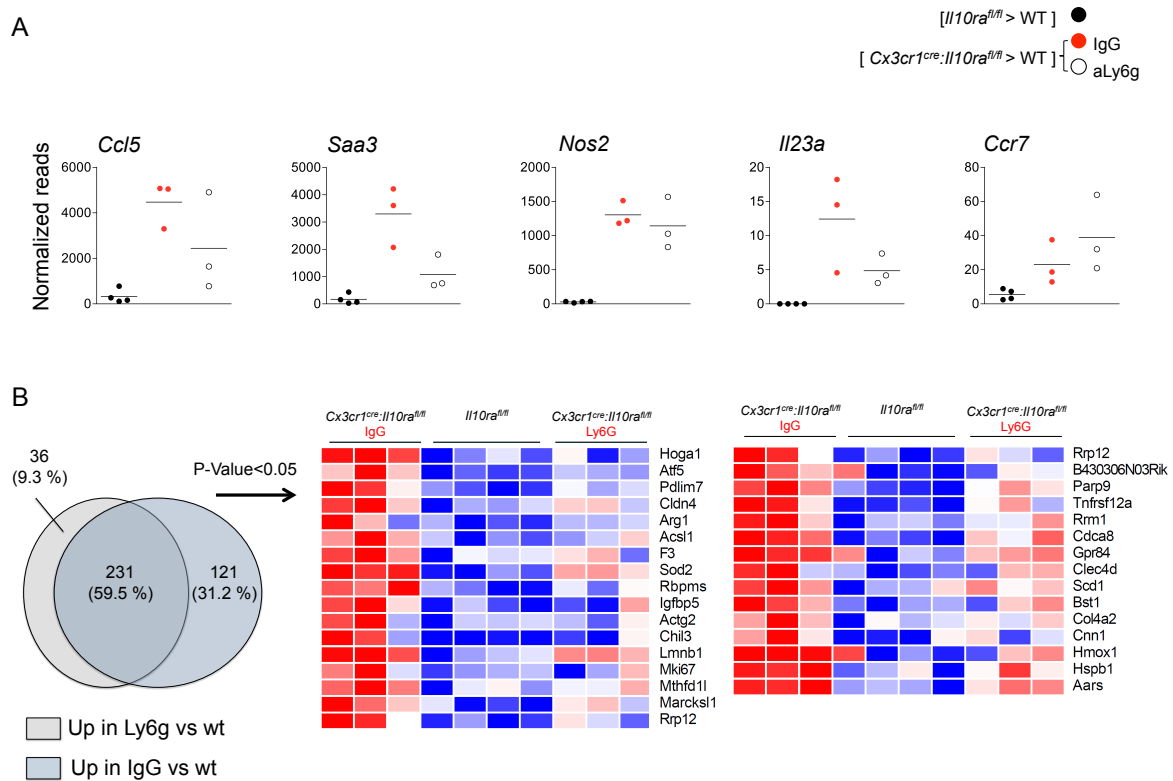**Supplementary Figure 6. Analysis of neutrophil elastase inhibitor (NEi) treated BM chimeras**

(A) Representative immunofluorescence images of the colonic tissue of [*Il10ra^{fl/fl}* > WT] or [*Cx3cr1^{cre}:Il10ra^{fl/fl}* > WT] BM chimeras**.**

(B) Schematic of NEi treatment

(C) Representative plots of flow cytometry analysis of the colonic *lamina propria* of [*Il10ra^{fl/fl}* > WT] or [*Cx3cr1^{cre}:Il10ra^{fl/fl}* > WT] BM chimeras left untreated or treated with NEi (left). Quantification of flow cytometry analysis (right). qRT-PCR analysis of *Nos2* expression in whole tissue extracts of colons of indicated BM chimeric mice (right).

(D) Representative plots of flow cytometry analysis of CD63 staining of neutrophils extracted from the colonic *lamina propria* of [*Cx3cr1^{cre}:Il10ra^{fl/fl}* > WT] BM chimeras left untreated or treated with NEi (left). Quantification of flow cytometry analysis (right).

Supplementary Figure 7. Analysis of transcriptomes of macrophages retrieved from BM chimeras treated with control IgG or neutrophil-depleting anti-Ly6G antibody.

(A) RNA-seq normalized read numbers for single genes of interest are plotted separately, each dot represents one mouse.

(B) Venn diagram and heatmap of RNAseq data of colonic macrophages extracted from [*Cx3cr1cre:Il10rafl/fl* > WT] BM chimeras treated with IgG control or anti Ly6G antibody. Normalized reads number were log transformed. Presented are genes of interest, significantly up-regulated in anti Ly6g treated vs WT control and unaltered in IgG treated vs WT.
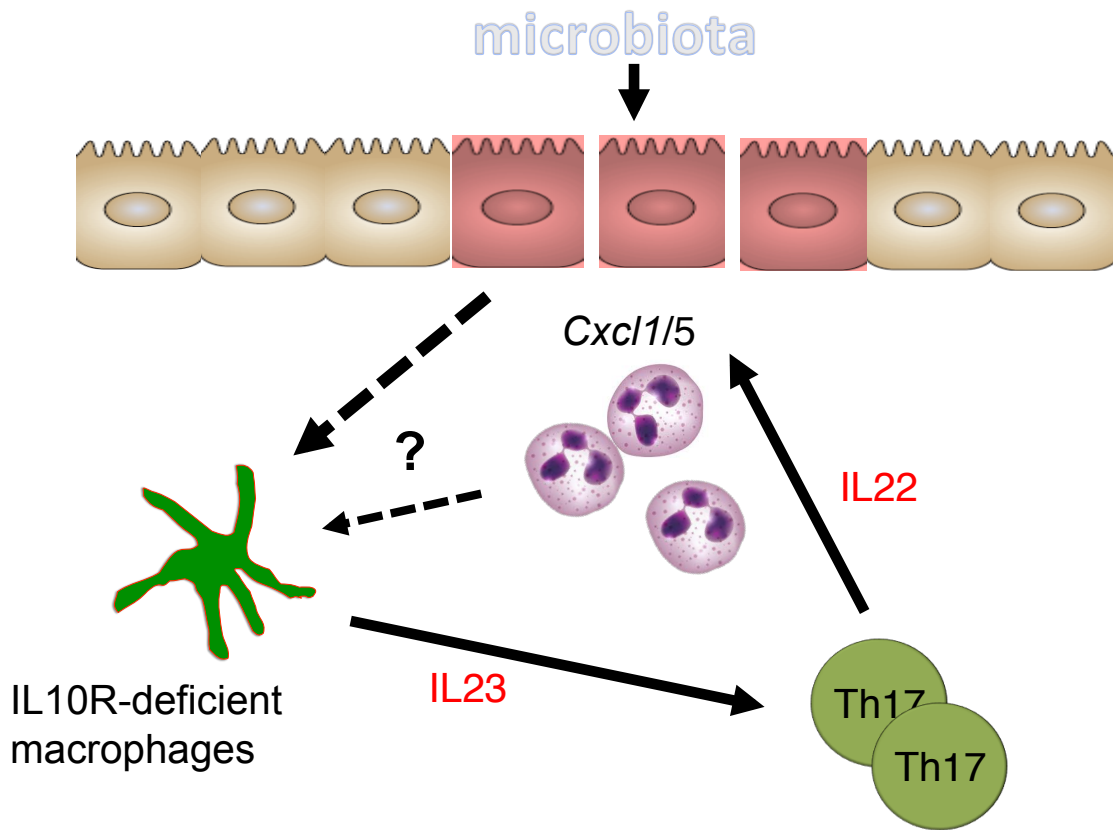
Supplementary Figure 8. Summary schematic illustrating the finding of this study.
In response to a microbial stimulus Il10R-deficient macrophage secrete IL-23 that induces
Th17 cells to produce Il-22. IL-22 in turn activates epithelial cells to produce chemokines that
recruit neutrophils that cause tissue damage. Our results suggest that the full pro-inflammatory
signature of macrophages is a secondary effect induced by epithelial response or the tissue
damage.