ONLINE SUPPLEMENTAL MATERIAL

Figure S1. Plexin B2 is required for the IFNγ response to DSS-induced colitis. Quantitative RT-PCR for IFNγ in distal colon of untreated or DSS-treated mice following 48hr *in vitro* culture in the presence or absence of anti-plexin B2 mAb. Data represents IFNγ mRNA expression relative to β-actin mRNA and is expressed in arbitrary units.

Figure S2. Normal architecture and integrity of the colon in CD100$^{-/-}$ mice. (**a**) CD3 immunofluorescence staining of untreated colon from WT and CD100$^{-/-}$ mice. Sections were counterstained with DAPI. (**b**) Alcian Blue/Nuclear Fast Red (top panels) and Periodic Acid/Schiff's Base (bottom panels) staining of untreated paraffin embedded sections of large intestine from WT, CD100-/- and γδTCR-/- animals. Digital images were acquired at 200x. Scale bar = 50μm

Figure S3. Severe DSS induced colitis in CD100-/- mice. (**a**) Criteria used in histological disease scores. (**b**) 3 days after removal of DSS, disease scores were assessed in WT and CD100$^{-/-}$ mice (mean $\pm$ SD, n=14 per group, *= $p < 0.05$ compared to WT). (**c**) Disease scores 14 days after removal of DSS (n=6 per group, * = $p < 0.05$ compared to WT).

Figure S4. IEL in CD100-/- mice are activated to produce TNFα following DSS-induced colitis. Intracellular FACS staining for TNFα in γδ IEL from WT and CD100-/- mice 2 days after removal of DSS. Cells are gated on γδTCR expression.

Figure S5. Altered IEL proliferation in CD100$^{-/-}$ mice. Proliferation of IEL in WT mice and CD100$^{-/-}$ mice as assessed by *in vivo* BrdU incorporation. (**a**) Percentage of γδ IEL incorporating BrdU in WT and CD100$^{-/-}$ mice was assessed by flow cytometry analysis (n=3, mean + SD, * = $p < 0.05$ compared to WT untreated). No significant difference was found between untreated and DSS-treated CD100$^{-/-}$ mice. (**b**) Representative example of BrdU incorporation by γδ IEL. Data is representative of three experiments. (**c**) Percentage of BrdU^{+} αβ IEL (mean ± SD of three separate experiments, * = $p < 0.05$ compared to WT untreated). (**d**) Representative example of BrdU incorporation by αβ IEL. Data is representative of three experiments.

Figure S6. γδ IEL from CD100-/- are not activated under homeostatic conditions. Intracellular FACS staining for TNFα, IFNγ and IL-17 in γδ IEL from untreated WT and CD100-/- mice. Cells are gated on γδTCR expression.

Figure S7. Acute inflammatory responses and apoptosis are not altered in CD100-/- animals. (a) Time course of myeloperoxidase (MPO) activity in the colon of WT and CD100-/- mice following DSS treatment. (b) Annexin V and propidium iodide staining of colon epithelial cells from WT and CD100-/- mice that were either untreated, or had been treated with DSS for 5 days and analyzed 3 days following the removal of DSS.

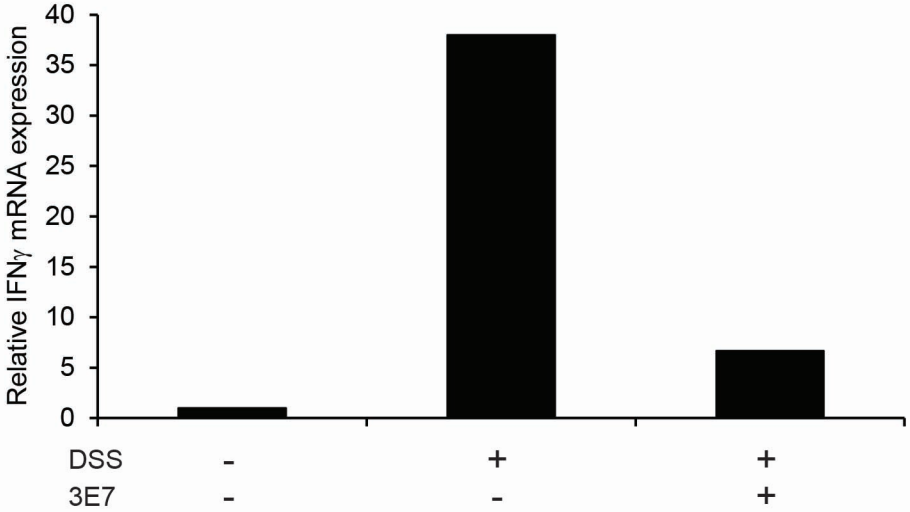
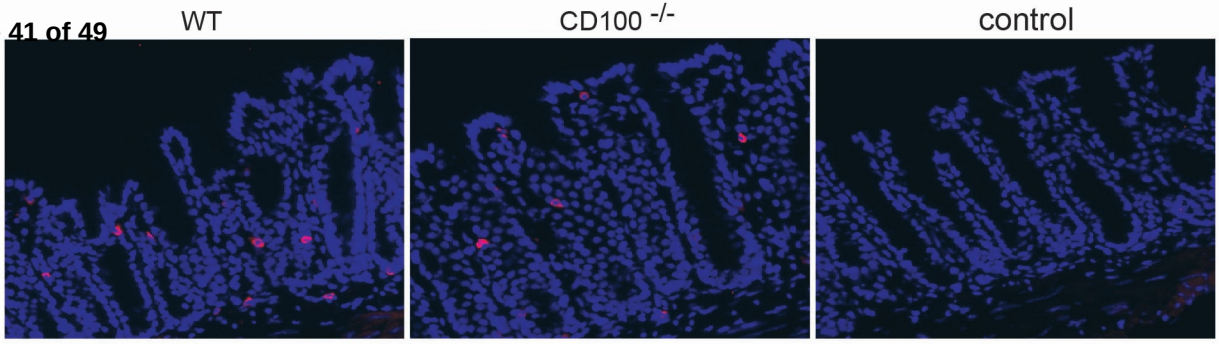


Figure S1



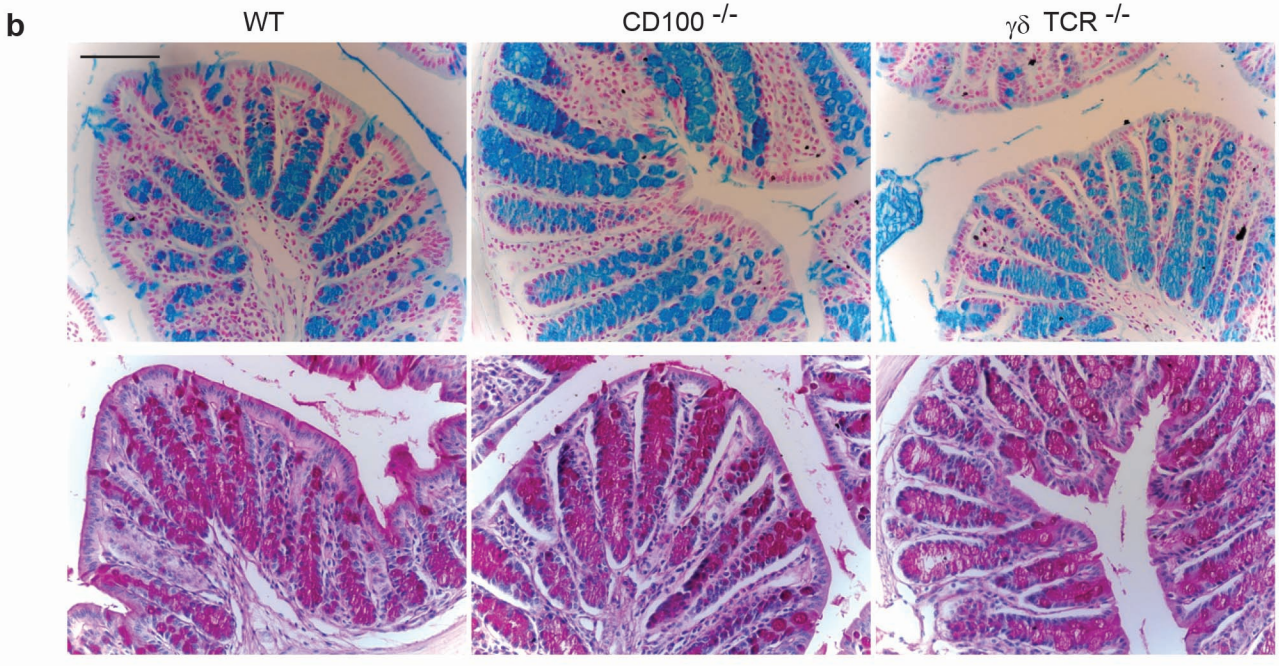
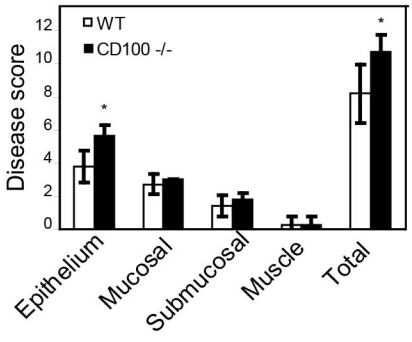


Figure S2

a

Epithelium	Mucosa	Submucosa
No Ulcer Present		
0 = Normal	**0** = None	**0** = None
1 = Hyperproliferation	**1** = Mild infiltrate	**1** = Mild infiltrate and/or edema
2 = 10-50% loss of crypt length	**2** = Moderate infiltrate	**2** = Severe infiltrate
3 = 50-90% loss of crypt length	**3** = Severe infiltrate	**Muscularis**
4 = Complete crypt loss		**0** = Not involved
Ulcer Present		**1** = Involved
5 = Ulcer less than 10 crypt widths		
6 = Ulcer greater than 10 crypt widths		

b



c

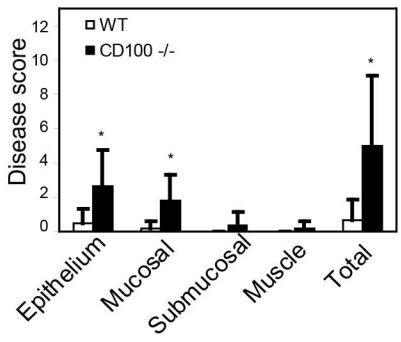


Figure S3

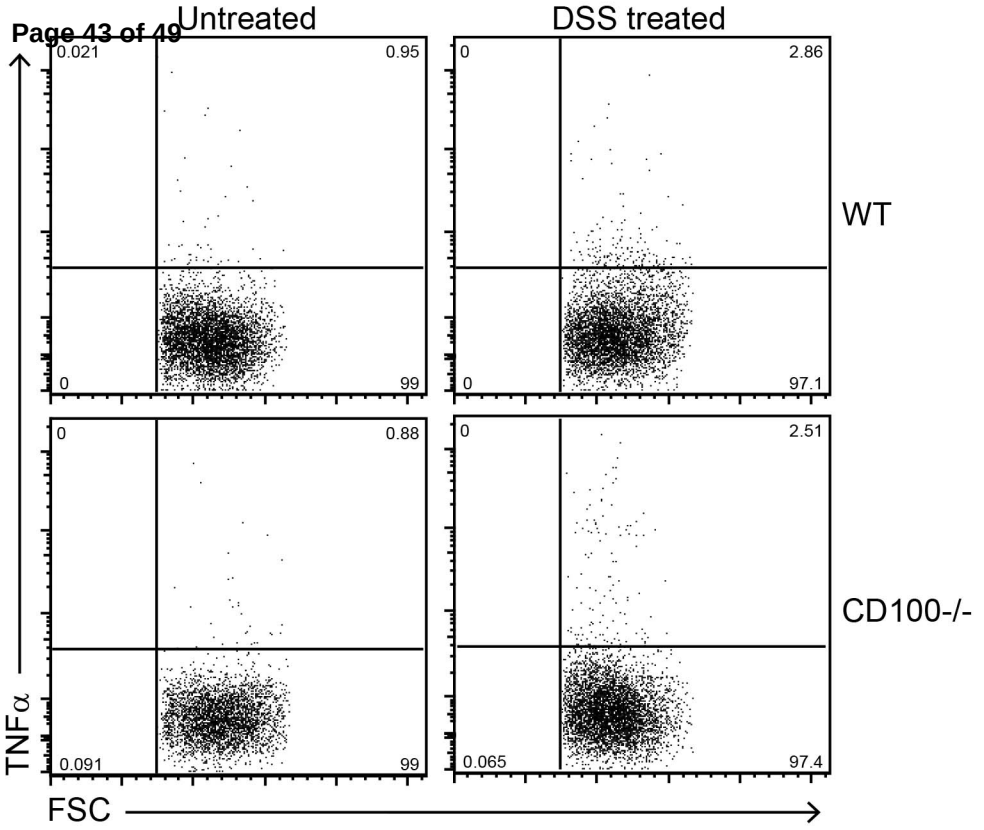
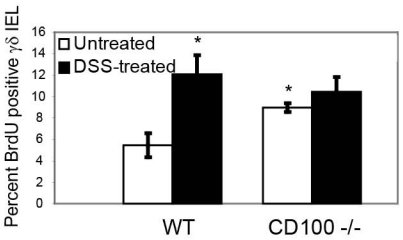
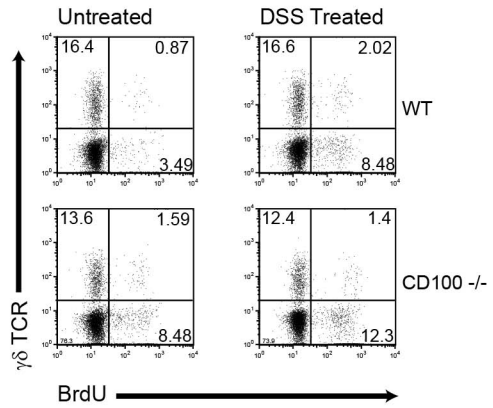

Untreated
DSS treated
0.021
0.95
0
99
0
2.86
0
97.1
WT
0
0.88
0.091
99
0
2.51
0.065
97.4
CD100-/-
TNFα
FSC

Figure S4

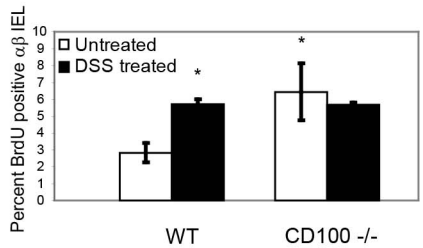
a



b



c



d

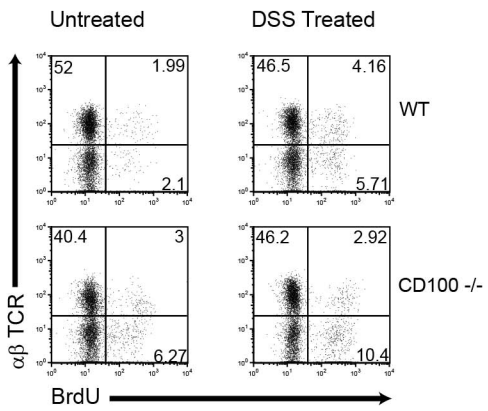


Figure S5

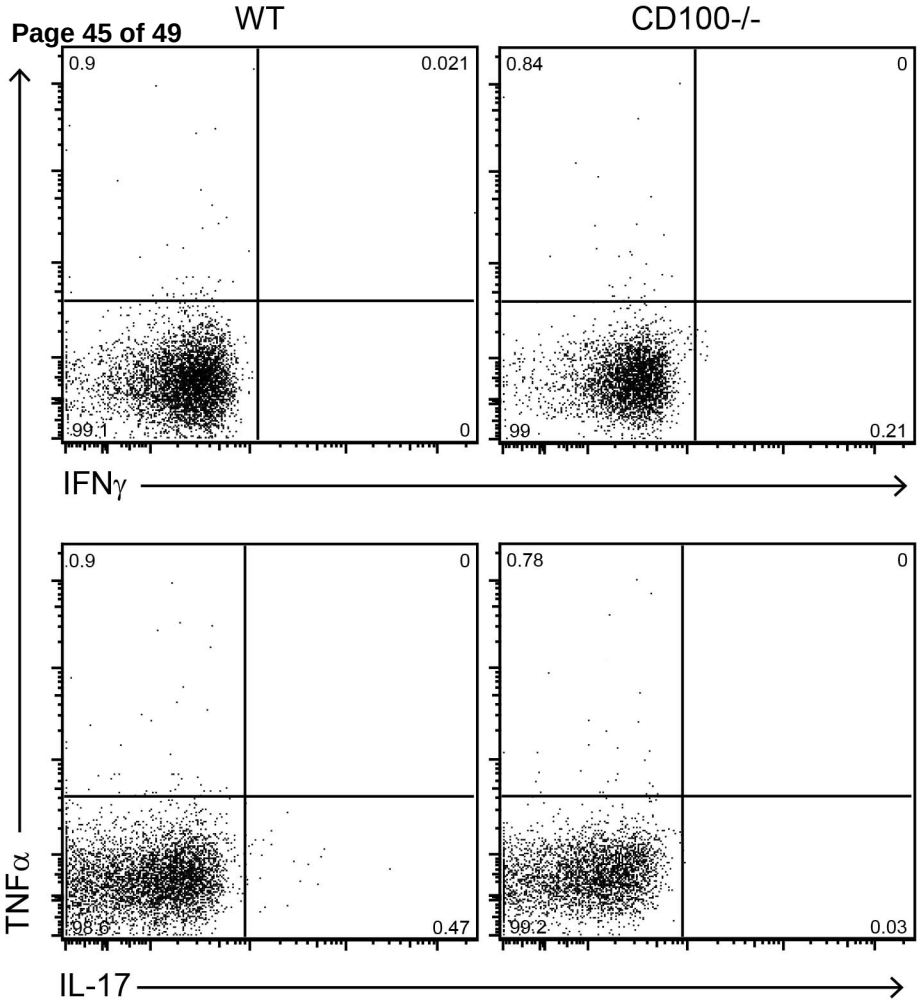
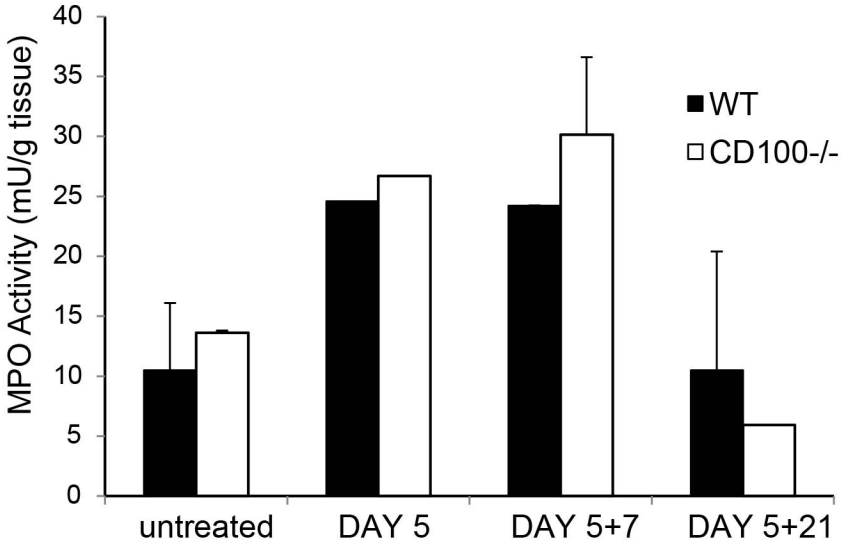

WT
CD100-/-
0.9
0.021
99.1
0
0.84
0
99
0.21
IFNγ
0.9
0
98.6
0.47
0.78
0
99.2
0.03
TNFα
IL-17

Figure S6

a


MPO Activity (mU/g tissue)
40
35
30
25
20
15
10
5
0
WT
CD100-/-
untreated
DAY 5
DAY 5+7
DAY 5+21

b

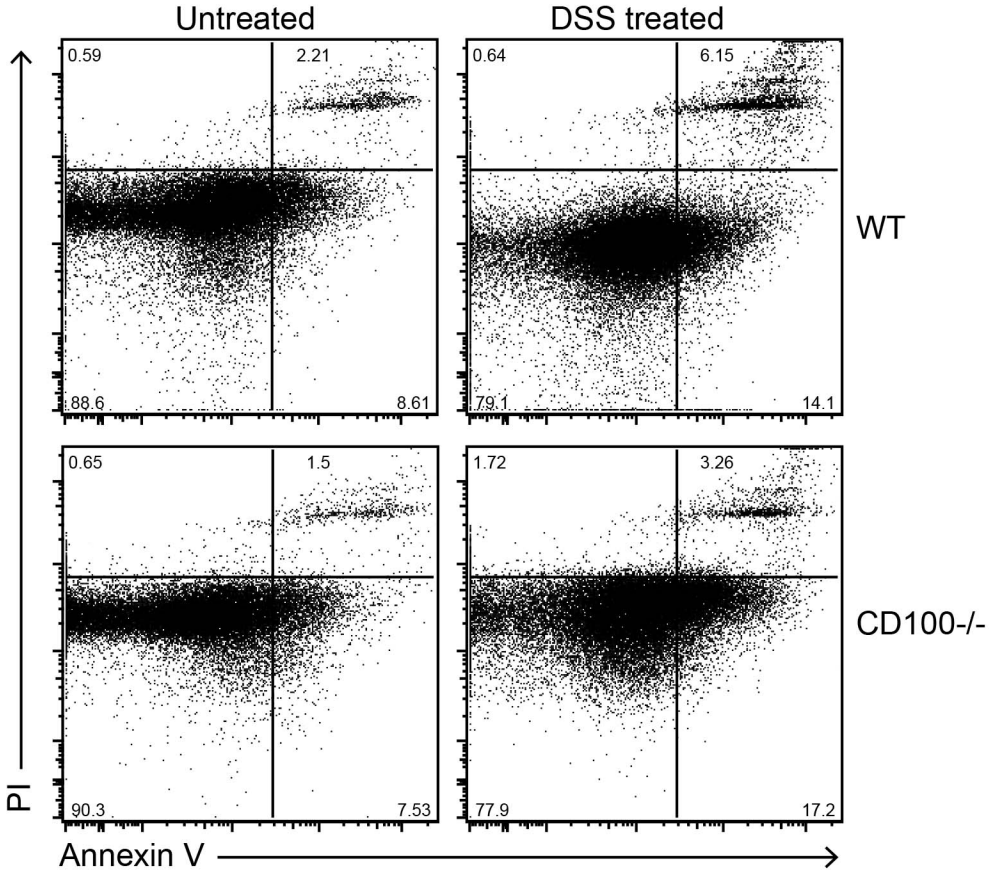

Untreated
DSS treated
0.59
2.21
88.6
8.61
0.64
6.15
79.1
14.1
WT
0.65
1.5
90.3
7.53
1.72
3.26
77.9
17.2
CD100-/-
PI
Annexin V

Figure S7