

SUPPLEMENTAL MATERIAL

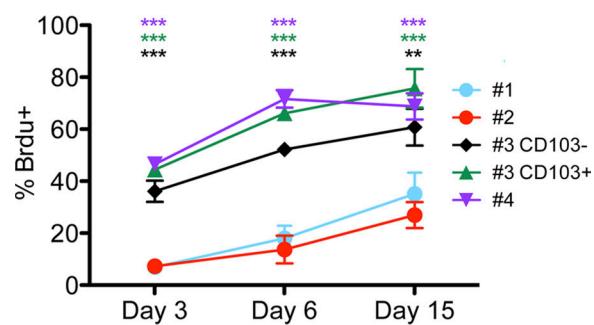
Rivollier et al., <http://www.jem.org/cgi/content/full/jem.20101387/DC1>

Figure S1. Subsets 1 and 2 have a much slower turnover than subsets 3 and 4. Percentages of BrdU⁺ cells within each defined cLP DC/MP subset in WT mice at various time points after continuous BrdU administration in drinking water. Each point on the graphs represents the mean \pm SEM of three mice per group. Unpaired Student's *t* tests were performed to compare subset 3 CD103⁻ (black statistics), subset 3 CD103⁺ (green statistics), or subset 4 (violet statistics) to both subsets 1 and 2, and the less significant p-values were plotted. **, P < 0.01; ***, P < 0.001.

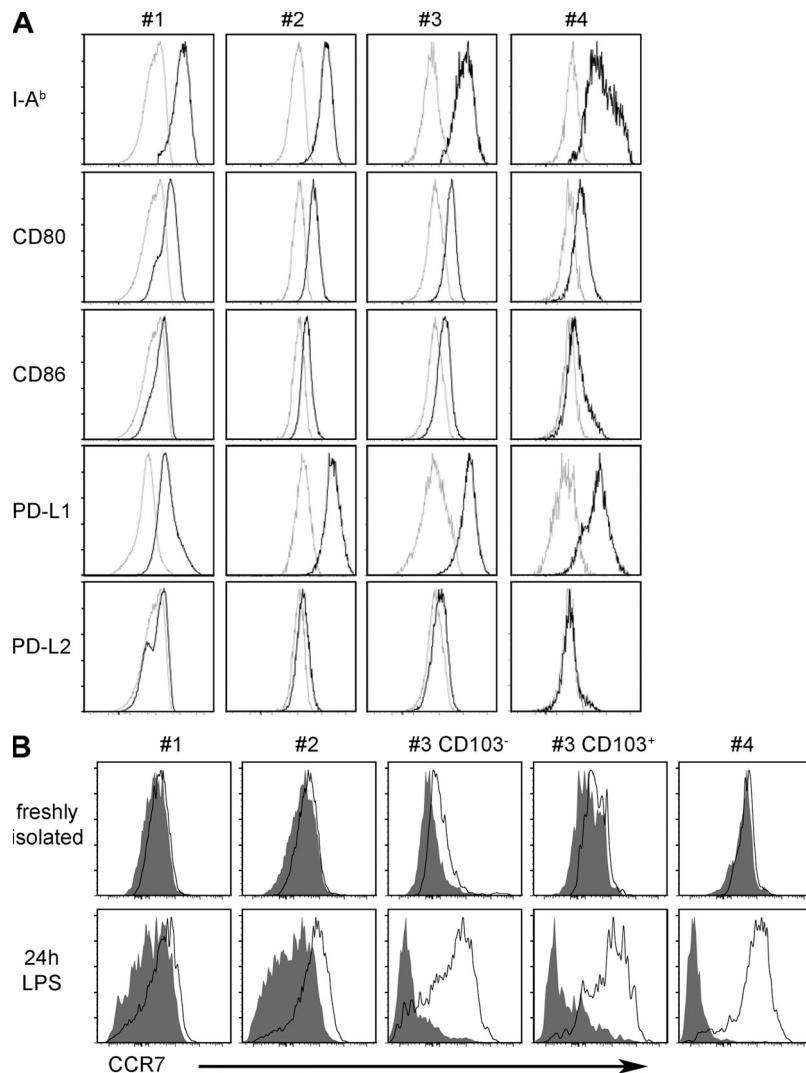


Figure S2. cLP subsets 3 and 4 but not 1 and 2 express CCR7 after *in vitro* activation. (A) Representative FACS analysis showing expression levels of I-A^b, CD80, CD86, PD-L1, and PD-L2 on subsets 1–4 from WT mice. All samples were pre gated on CD45⁺MHC-II^{hi}lin⁻ cells before the expression of each marker was analyzed on each specific phagocyte subset (black lines) compared with the appropriate isotype controls (gray lines). (B) Representative FACS analysis showing CCR7 surface expression (black lines) and isotype controls (gray-filled areas) on WT subsets 1–4 freshly isolated from the cLP or after 24 h of culture in the presence of 1 µg/ml LPS. Results are representative of at least two experiments with one to three colons each.

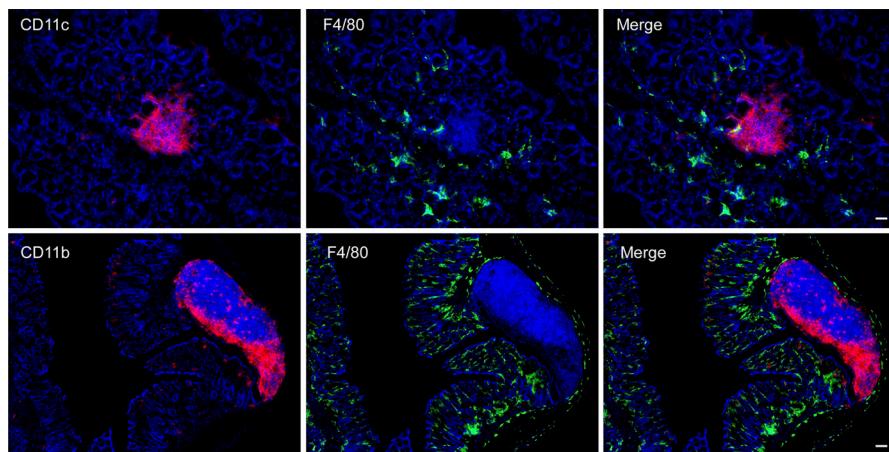


Figure S3. F4/80⁺ cells are excluded from the organized lymphoid structures of the cLP. In situ expression of CD11c (red) and F4/80 (green; top) or CD11b (red) and F4/80 (green; bottom) on C57BL/6 mouse colon sections. Hoechst counterstain is blue. Data are from two individual mice and are representative of >10 mice. Bars, 50 μ m.

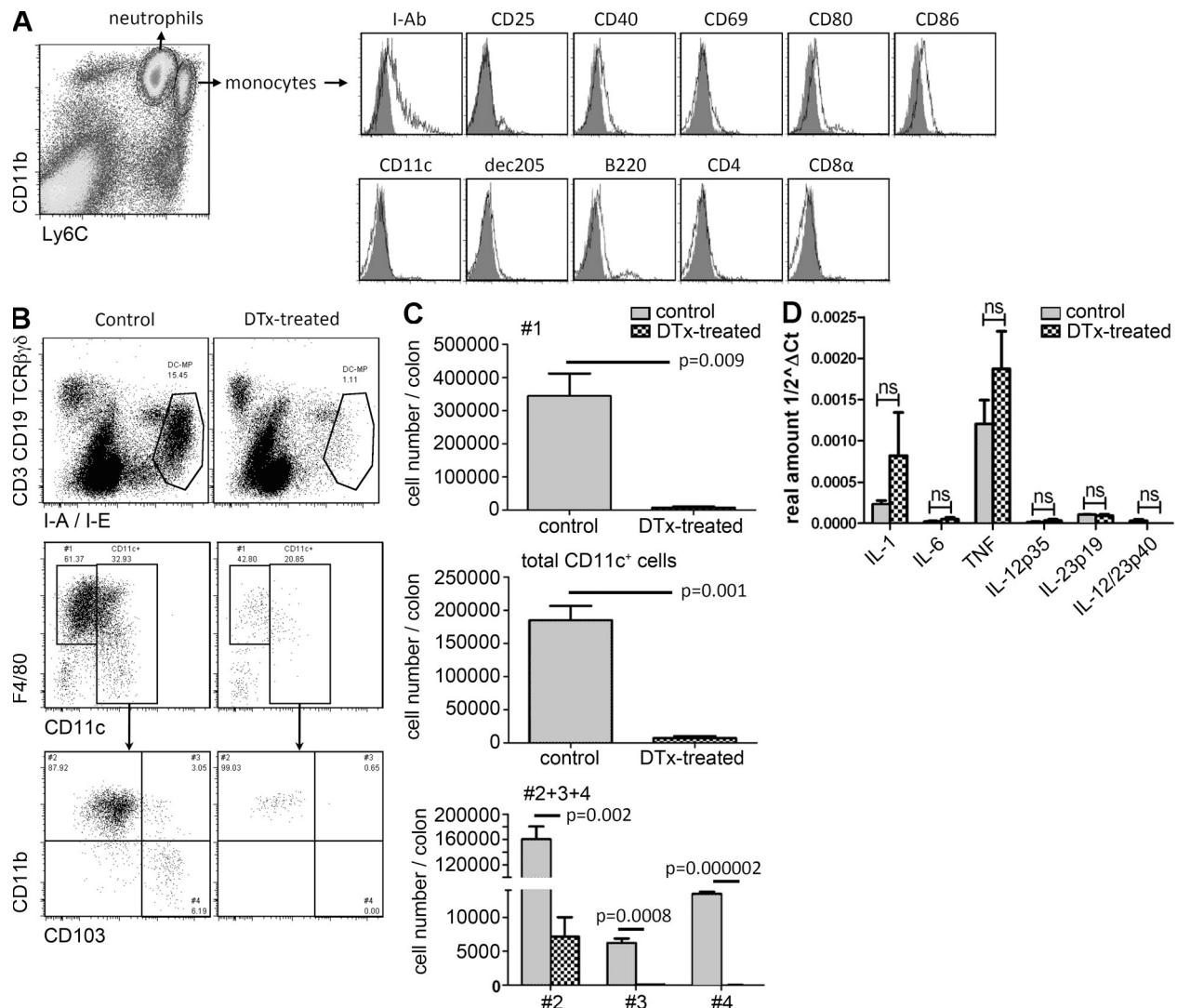


Figure S4. DTx treatment efficiently depletes cLP subsets 1–4 without inducing significant inflammation in the colon of CD11cDTR → C57BL/6 chimeric mice. (A) FACS analysis of the indicated surface markers (black lines) and their respective isotype controls (gray-filled histograms) on bone marrow CD11b⁺Ly6C^{hi} M0s. Plots are representative of at least two experiments with 1–10 pooled bone marrows. (B) FACS analysis showing the MHC-II/Iin (top), CD11c/F4/80 (middle) and CD11b/CD103 (bottom) expression profiles in the cLP of CD11cDTR → C57BL/6 mice 24 h after the i.p. injection of DTx (DTx treated) or PBS (control). (C) Quantification of the absolute numbers of cLP subsets 1–4 and total CD11c⁺ cells (gated as shown in A) in DTx-treated or control animals. Results are mean \pm SD of three mice per group. (D) Real-time RT-PCR analysis of inflammatory cytokine mRNA expression in total cLP cells from CD11cDTR → C57BL/6 mice 24 h after DTx (DTx treated) or PBS (control) injection. Results have been normalized to GAPDH and are presented as mean real amounts ($1/2\Delta^{\text{Ct}}$) \pm SD of three mice per group.

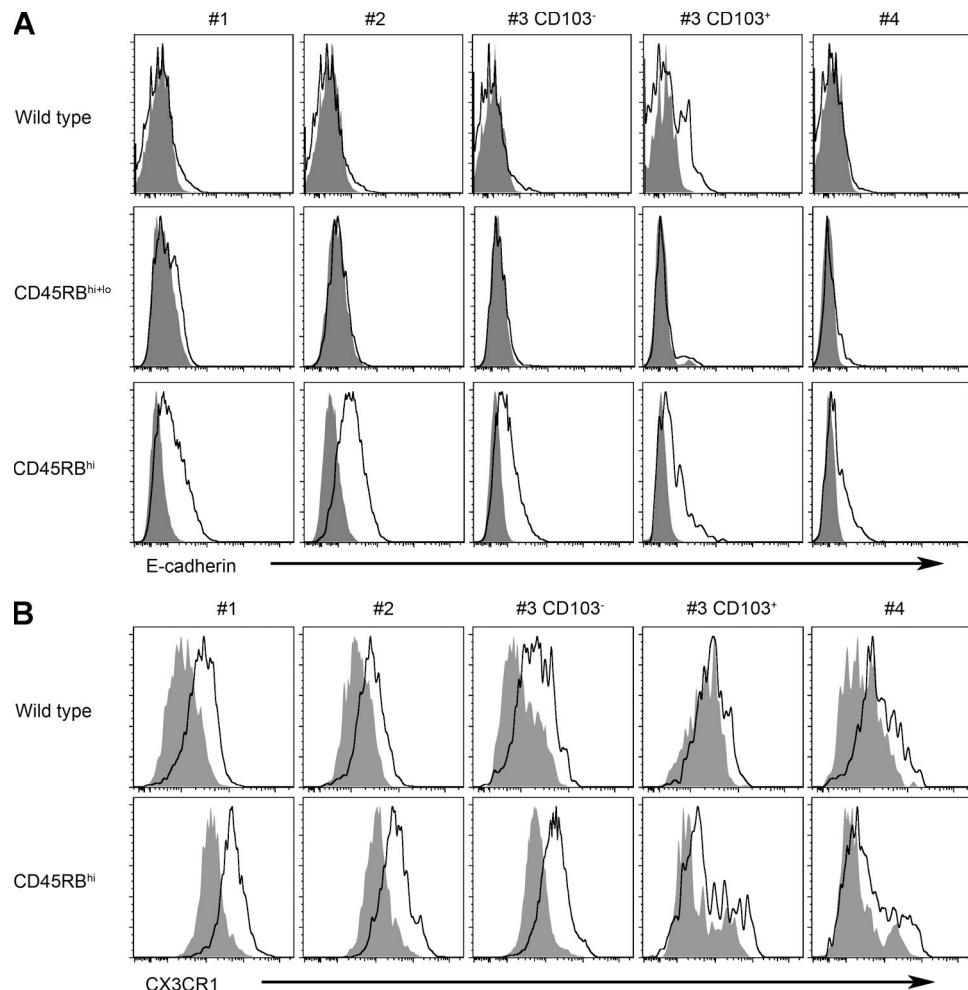


Figure S5. The MP subsets 1 and 2 and also the CD103⁻ DC subset 3 express E-cadherin and CX3CR1 during colitis. (A) FACS analysis of E-cadherin surface expression (black lines) on cLP subsets 1, 2, 3 CD103⁻, 3 CD103⁺, and 4 from WT, colitic (CD45RB^{hi}) and control (CD45RB^{hi/+lo}) mice. Iso-type controls are shown as gray-filled histograms. Histograms are representative of two independent experiments with 3–10 pooled colons. (B) FACS analysis of CX3CR1 surface expression (black lines) on cLP subsets 1, 2, 3 CD103⁻, 3 CD103⁺, and 4 from WT and colitic (CD45RB^{hi}) mice after staining with anti-CX3CR1 rabbit polyclonal antibody. Control staining using purified rabbit IgG is shown by the gray-filled histograms. Histograms are representative of one experiment with three independent mice.

Table S1. References of the Applied Biosystems FAM-labeled primers used in this study

| Gene | Assay ID |
|--------------|---------------|
| GAPDH | Mm99999915_g1 |
| <i>Il10</i> | Mm99999062_m1 |
| <i>Il12a</i> | Mm99999066_m1 |
| <i>Il12b</i> | Mm99999067_m1 |
| <i>Il23</i> | Mm00518984_m1 |
| <i>Il27</i> | Mm00461164_m1 |
| <i>Il6</i> | Mm99999064_m1 |
| <i>Inos</i> | Mm00440485_m1 |
| <i>Tgfb1</i> | Mm00441724_m1 |
| <i>Tgfb2</i> | Mm00436952_m1 |
| <i>Tgfb3</i> | Mm00436960_m1 |
| TNF | Mm00443258_m1 |

Table S2. Classification of colonic MPs and DCs according to their surface marker expression and proposed functions

| Subset name | Markers | | | | | Function |
|----------------------|---------|-------|--------|-------|-------|--|
| | F4/80 | CD11c | CX3CR1 | CD11b | CD103 | |
| MPs | | | | | | |
| 1 | +++ | — | +++ | +++ | — | Inflammation dampening via IL-10 |
| 2 | +++ | +++ | +++ | +++ | — | Inflammation dampening via IL-10 |
| DCs | | | | | | |
| 3 CD103 [−] | ++ | +++ | ++ | +++ | — | Drives Th1 inflammation |
| 3 CD103 ⁺ | ++ | +++ | — | +++ | +++ | Induces foxp3 ⁺ T reg cell conversion |
| 4 | — | +++ | — | — | +++ | ND |