**Figure S1. Location and accumulation of BrdU^+ nuclei and nuclear doublets in 2-wk lesions.** (a–c) En face confocal microscope images of the ascending arch LC harvested 2 h after BrdU pulse labeling from Ldlr^{−/−} mice fed CRD for 2 wk and stained for BrdU (green), lipid (red), and nuclei (blue). Images from xz (a and b) or yz (a) planes show subendothelial localization of the BrdU^+ nuclei. b is a z-stacked image of the lesion. Dashed lines indicate the locations in the intimal layer where the images were analyzed in the z plane. c shows examples of BrdU^+ cells that are in contact with lipid-laden foam cells (arrowheads). (d) The abundance of BrdU^+ cells in the LC of the ascending arch containing 2-wk atherosclerotic lesions was determined by en face confocal microscopy at 1–6 h after BrdU injection. This time window was selected because BrdU-labeled monocytes are not released from the BM within 6 h of pulse labeling; thus, monocyte recruitment cannot contribute to the number of BrdU^+ nuclei in the aortic intima. (e) En face confocal images of the ascending arch LC harvested 2 h after BrdU pulse labeling from Ldlr^{−/−} mice fed CRD for 2 wk and stained for BrdU (green), lipid (red), and nuclei (blue). BrdU^+ nuclear doublets are marked with arrowheads and likely represent daughter cells. (f) Percentage of BrdU^+ nuclei that appear as doublets at 1–24 h after BrdU injection. All images are representative of at least four independent experiments. Data in d and f were derived from four independent experiments. Means ± SEM at the 1, 3, 6, and 24 h time points were derived from four mice per time point and at 2 h from seven mice. Bars, 20 μm.
Figure S2. PTx specifically blocks monocyte recruitment into thioglycollate-inflamed peritoneal cavity and increases circulating blood leukocytes. (a and b) Representative flow cytometry plots of gated peritoneal exudate cells (a) or unfixed leukocytes obtained from blood (b) harvested 24 h after i.p. thioglycollate injection of control and PTx-treated mice. PTx was injected i.v. concurrently with thioglycollate. The relative abundance (percentage) of Ly-6C<sup>high</sup> and Ly-6C<sup>low</sup> monocytes (CD115<sup>+</sup>) and neutrophils (Ly-6C<sup>+</sup>CD115<sup>+</sup>) is indicated next to corresponding circles. (c and d) The percentage and absolute number of CD115<sup>+</sup>Ly-6C<sup>high</sup> and CD115<sup>+</sup>Ly-6C<sup>low</sup> monocytes, neutrophils, and B lymphocytes in thioglycollate-elicited peritoneal cells (c) and in blood (d) harvested from control or PTx-treated mice is shown. For each leukocyte type, absolute numbers (means ± SD) were derived by multiplying the total leukocyte count (determined using a hemocytometer) by the relative abundance for each leukocyte type (determined by flow cytometry). Neutrophils were also assessed by cytospins (c) and blood films (d). The number of mice per group (n) is indicated, as are p-values when comparing differences between control and PTx groups. Note that PTx reduced the absolute number of peritoneal leukocytes. The percentage and absolute number of peritoneal CD115<sup>+</sup>Ly-6C<sup>high</sup> and CD115<sup>+</sup>Ly-6C<sup>low</sup> monocyte subsets were reduced, but not of neutrophils (Ly-6C<sup>+</sup>CD115<sup>+</sup>) or B cells (CD19<sup>+</sup>). In contrast, PTx treatment increased the absolute number of circulating leukocytes 6-fold and neutrophils by >15-fold. Circulating Ly-6C<sup>high</sup> and Ly-6C<sup>low</sup> monocytes were also increased, but their relative abundance did not change. In a and b, representative data from two independent experiments are shown. In each experiment, two or three pairs of control and PTx-treated mice were studied for a total of five mice per group. Peritoneal exudates were analyzed in all mice, as was the blood in four mice per group. Analysis of B cells (c) and blood film analysis for neutrophils (d) was performed in two mice per group. FS, forward scatter; SS, side scatter.
Figure S3. Topographic distribution of CD11c, CD68, and CD11b expression in early atherosclerotic lesions. (a and b) Ldlr<sup>−/−</sup> mice fed a CRD for 2 wk were injected with BrdU at 2 h before harvesting of the ascending aorta. Immunostaining for BrdU (green) was performed in conjunction with CD11c (yellow) and CD68 or CD11b (red), and tissues were examined by en face confocal microscopy. Nuclei are blue. The majority of foam cells present in the LC of the arch of 2-wk-old lesions expressed CD11c and CD68 (a), whereas CD11b<sup>+</sup> cells were located in clusters (b). In four independent experiments, four mice were costained for BrdU, CD68, and CD11c, and four mice were costained for BrdU, CD11b, and CD11c. A representative mouse in each group was selected and composite images were assembled. Bars, 80 μm.
Figure S4. Proliferating CD11c+ intimal cells coexpress MHC II and CD68. (a and b) Ldlr−/− mice fed a CRD for 2 wk were injected with BrdU at 2 h before harvesting of the ascending aorta. Immunostaining for BrdU (green) was performed in conjunction with CD11c (yellow) and MHC II or CD68 (red). Nuclei are blue. En face confocal microscopy images showing MHC II (a) and CD68 (b) costaining in the LC are shown. Note that the majority of proliferating (BrdU+) intimal cells expressed CD11c as well as MHC II and CD68 (arrowheads). Representative images from two out of four mice stained for MHC II and one out of four mice stained for CD68 are shown. Four independent experiments were performed (two MHC II costaining experiments and two CD68 experiments). Bars, 20 μm.