Supporting Information

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SI Materials and Methods

Experimental Animals. Nlrp3$^{-/-}$ (Nlrp3tm1Bhk/J) and CD19$^{-/-}$ [B6.129P2(C)-Cd19tm1(cre)Cgn/J] mice were obtained from Jackson Laboratories. Animals were kept and bred under specific pathogen-free conditions at the animal facility of the Department of Microbiology, Tumor and Cell Biology. All experiments were approved by the local ethical committee (North Stockholm district court).

Injections. CD19$^{-/-}$ mice were injected with IL-18, as previously described (1). Briefly, mice were injected with 2 μg rmIL-18 (Reprokine Ltd) intraperitoneally daily for 9 d.

Cell Isolation and Cell Culture. Splenocytes and peritoneal lavage cells from six 30–45-wk-old female Apoe$^{-/-}$ mice were enriched for B cells using MACS purification and stained with markers for the MZB, FOB, and B1a subsets (see Flow Cytometry and Table S1). Cells from individual mice were pooled, and FOB and MZB were sorted from spleen and B1a cells from the peritoneal lavage, using a FACSAria Fusion cell sorter. The sorted FOB, MZB, and B1a cells were placed in cell culture in complete RPMI (350,000 cells/200 μL per 96 wells) and stimulated with LPS (10 μg/mL), and supernatants were collected 6 d later.

Adoptive Transfer. Splenocytes were enriched for B cells by MACS, and MZB and FOB were sorted on a MoFlow and pooled from 14 old Apoe$^{-/-}$ mice, and 1.9–2 × 10^6 sorted cells were transferred to 5–6 young (6–8-wk-old) Apoe$^{-/-}$ mice.

Confocal and Reflection Microscopy. Spleens from 23-wk-old Apoe mice were cut in 6-μm-thick sections in a cryostat microtome, blocked in 5% (vol/vol) goat serum, stained with Nile red and F4/80, and mounted with 90% (vol/vol) glycerol. A heart section with lesions was used for control. To image cholesterol crystals, we combined confocal reflection microscopy with fluorescence microscopy on a Leica SP2 Acousto-Optical Beam Splitter (AOE) confocal laser-scanning microscope. Reflection was captured by setting the AOBS of the 488-nm laser excitation line to reflection mode, and the detector channel was placed directly over the wavelength of the selected laser channel for reflection light capture. The AOBS microscope was set to allow 10% of laser light into the collection channel. Fluorescence was captured by standard confocal imaging techniques, and images were obtained by sequential scanning of the respective excitation lasers and detectors (2).

1. Enoksson SL, et al. (2011) The inflammatory cytokine IL-18 induces self-reactive innate antibody responses regulated by natural killer T cells. *Proc Natl Acad Sci USA* 108(51): E1399–E1407.

2. Hornung V, et al. (2008) Silica crystals and aluminum salts activate the NALP3 inflammasome through phagosomal destabilization. *Nat Immunol* 9(8):847–856.

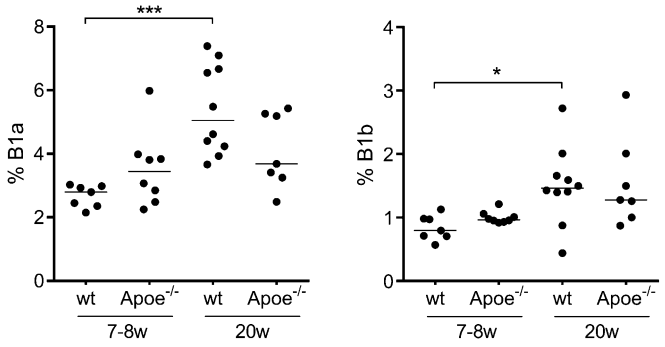

Fig. S1. Peritoneal B1 cells increase with age. B1a (*Left*) and B1b (*Right*) in the peritoneal cavity of 7–8-wk-old (7-8w) and 20-wk-old C57BL/6 (wt) and Apoe$^{-/-}$ mice were investigated by flow cytometry. Median and individual mice (n = 7–10) are plotted. *P < 0.05, ***P < 0.001 by Mann–Whitney U test.

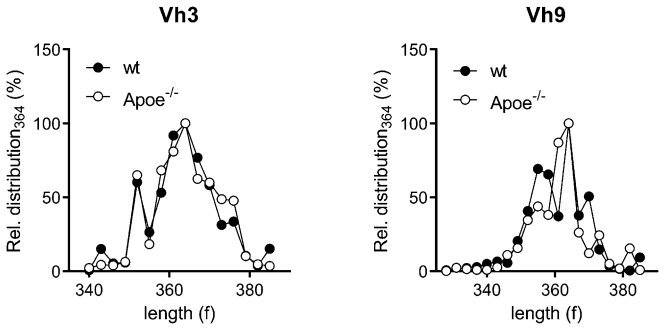

Fig. S2. Clonal expansion was studied through spectratyping of splenocytes from 20-wk-old C57BL/6 (wt) and Apoe$^{-/-}$ mice. The length (in nucleotides) of each detected fragment represents a clone, and the spectrum yielded of respective measured Vh family represents the clonal distribution. To account for experimental variation, we first normalized the area of each measured peak to an arbitrary peak of respective Vh family before comparing the relative clonal distribution (Rel.dist.) between the groups. Shown are Vh3 (*Left*) and Vh9 (*Right*). Each data point represents the median of eight individual mice.

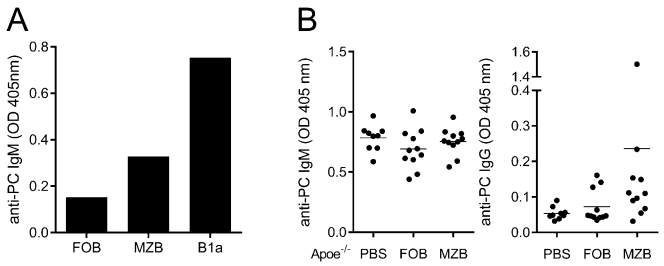

Fig. S3. MZB are capable of producing anti-PC antibodies. FOB, MZB from the spleen, and peritoneal B1a cells were sorted from old Apoe$^{-/-}$ mice and stimulated in vitro with 10 μg/mL LPS for 6 d, and anti-PC IgM was measured in the supernatants by ELISA (*A*). FOB or MZB were sorted from old Apoe$^{-/-}$ mice and transferred to young Apoe$^{-/-}$ mice. Anti-PC IgM and IgG was measured in the serum 10 wk after transfer by ELISA (*B*). Data from one experiment in which cells from six mice were pooled (*A*) or pooled from two experiments. Mean and individual mice (n = 9–11) are shown (*B*).

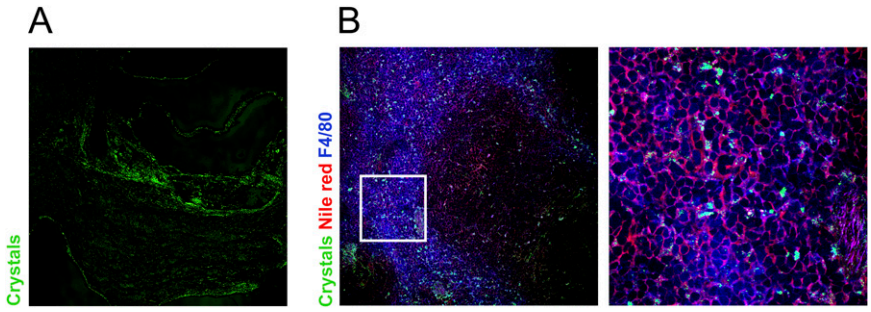

Fig. S4. Cholesterol crystals are present in the spleen of old Apoe$^{-/-}$ mice. Crystals were visualized in the spleen of old Apoe$^{-/-}$, using reflection microscopy (*B*). Nile red and F4/80 were used to stain lipid accumulation and macrophages, respectively. The boxed area is shown at higher magnification on the right. Aortic lesions in the aortic root of a heart were used as a positive control (*A*).

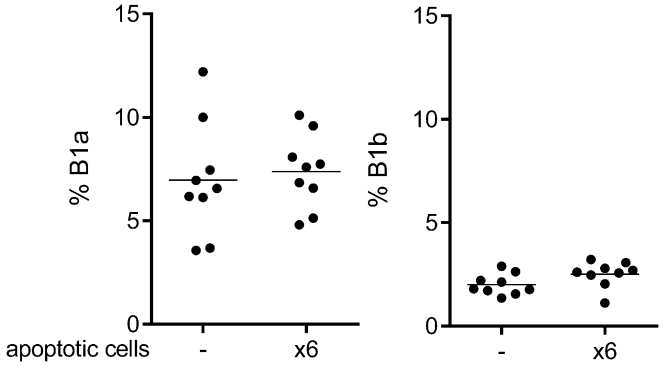

Fig. S5. Peritoneal B1 cells remain unchanged after apoptotic cell injections. B1a (*Left*) and B1b (*Right*) in the peritoneal cavity of WT mice injected six times (×6) i.v. with apoptotic cells and uninjected controls (–) were investigated by flow cytometry. Mean and individual mice (n = 9) are plotted. Mean and individual mice are shown, n = 9. Data are representative of two independent experiments.

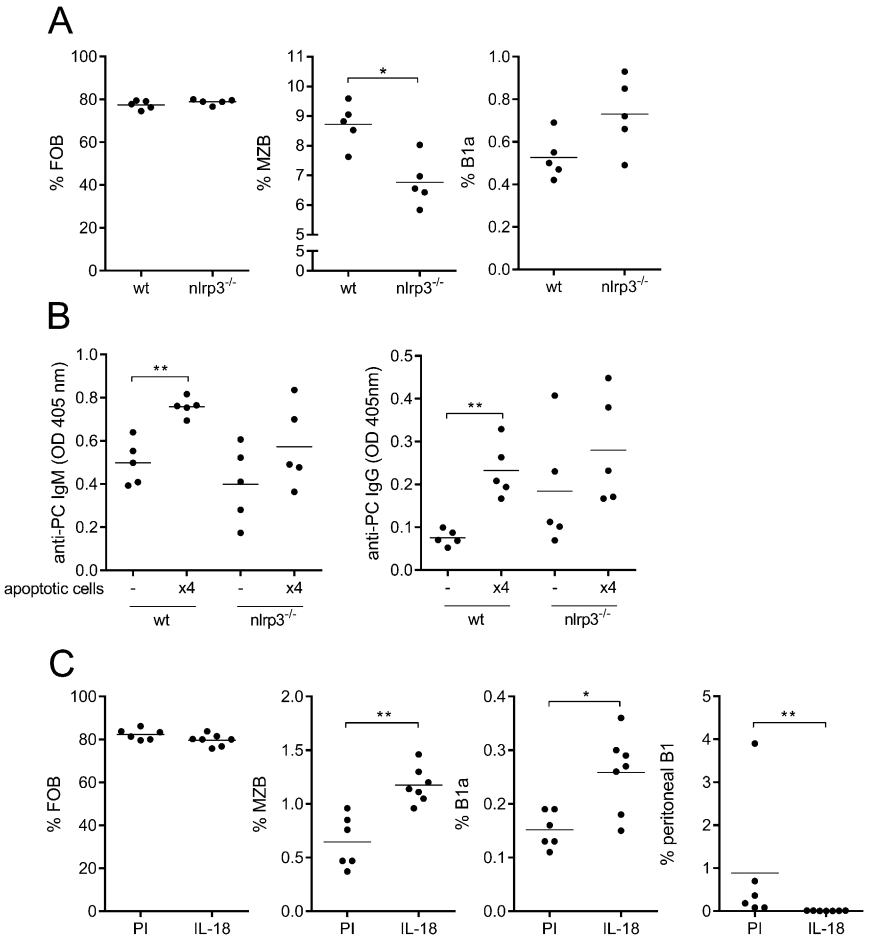

Fig. S6. The inflammasome drives an anti-PC antibody response and MZB expansion. Spleens of noninjected (–) and apoptotic cell-injected (×4) nlrp3$^{-/-}$ and C57BL/6 (WT) mice were investigated for FOB, MZB, and B1a cells by flow cytometry (*A*), and anti-PC IgM and IgG was measured by ELISA (*B*). Spleens of noninjected (PI) and IL-18–injected WT mice were investigated for FOB, MZB, and splenic and peritoneal B1a cells by flow cytometry (*C*). Mean and individual mice (n = 5–7) are plotted. Data shown from one experiment. *P < 0.05, **P < 0.01, and ***P < 0.001 by a Mann–Whitney U test.

Table S1. B-cell subsets and their defining surface markers

B-cell subset	Abbreviation	Surface markers
Peritoneal B1a	B1a	$CD19^{hi}$ $B220^{lo}$ $CD11b^{+}$ $CD5^{+}$
Peritoneal B1b	B1b	$CD19^{hi}$ $B220^{lo}$ $CD11b^{+}$ $CD5^{-}$
Splenic B1a	B1a	$CD19^{hi}$ $B220^{lo}$ $CD5^{+}$
Marginal zone B cell	MZB	$B220^{+}$ $CD21^{hi}$ $CD23^{lo}$
Follicular B cell	FOB	$B220^{+}$ $CD21^{lo}$ $CD23^{+}$
Germinal center B cell	GC B cell	$B220^{+}$ IgD^{lo} $CD95^{+}$ $GL7^{+}$
Antibody-forming cell	AFC	$B220^{lo}$ $CD138^{+}$