SUPPLEMENTAL MATERIAL

DETAILED METHODS

Human subjects

Patients were recruited for study through the Cardiac Catheterization laboratory at the University of Virginia as previously described[1]. All participants provided written informed consent prior to enrollment, and the study was approved by the Human IRB Committee at UVA. A total of 50 patients presenting for a medically indicated diagnostic cardiac catheterization were enrolled if they met inclusion criteria and had a vessel suitable for intravascular ultrasound (IVUS). All were outpatients with stable coronary syndrome. Patients were excluded if they had: any acute illness, type 1 diabetes, current acute coronary syndrome, autoimmune disease or on immunosuppressive therapy, prior organ transplantation, anemia, pregnancy, HIV infection, or no vessel suitable for IVUS. No patient was on anticoagulation or had deep vein thrombosis or pulmonary embolism.

IVUS

One-vessel IVUS was performed on 50 participants in accordance with the American College of Cardiology standards for image acquisition on a non-infarct related artery as previously described[1]. Summarily, a 2.6 F, 30 MHz IVUS catheter (Volcano Corporation) was advanced over a guidewire into either the left anterior descending coronary artery or the least-angulated vessel providing the longest length for evaluation. Images were obtained at 30 frames/s for a minimum of 40 mm of vessel. IVUS images

were analyzed using virtual histology by two blinded investigators according to the guidelines by the American College of Cardiology. Every 60th image was analyzed at 1 mm intervals for a total of 40 mm along the length of the artery beginning at the proximal end. Volcano Image Analysis Software was used to define plaque tissue composition into fibrous, necrotic, or calcified areas, which were expressed as a percentage of total intima area. Atheroma burden and maximum stenosis were quantified as previously described[1].

Systemic cholesterol measurements

Human arterial blood samples were obtained just before IVUS and blood was collected in BD vacutainer serum tubes, centrifuged at 400 rcf for 10 minutes, and then serum was aliquoted into a new tube. Human serum cholesterol quantification was performed by the University of Virginia Medical Laboratories as previously described[2].

Mice

All animal protocols were approved by the Animal Care and Use Committee at the University of Virginia. CXCR4$^{fl/fl}$ and ApoE$^{-/-}$ mice were purchased from Jackson Laboratory. CD19$^{cre/+}$ and Rag1$^{-/-}$ mice were provided by Dr. Timothy Bender (University of Virginia). CXCR4$^{fl/fl}$ mice were bred to the ApoE$^{-/-}$ line and then to CD19$^{cre/+}$ mice to develop CXCR4$^{fl/fl}$ ApoE$^{-/-}$ CD19$^{cre/+}$ mice. These mice were then bred to CXCR4$^{fl/fl}$ ApoE$^{-/-}$ CD19$^{+/+}$ mice to generate CXCR4$^{fl/fl}$ ApoE$^{-/-}$ CD19$^{cre/+}$ (CXCR4^{BKO} ApoE$^{-/-}$) and CXCR4$^{fl/fl}$ ApoE$^{-/-}$ CD19$^{+/+}$ (CXCR4^{WT}ApoE$^{-/-}$) littermate controls. Rag1$^{-/-}$ or sIgM$^{-/-}$ mice

were crossed with ApoE$^{-/-}$ mice to generate Rag1$^{-/-}$ApoE$^{-/-}$ or sIgM$^{-/-}$ApoE$^{-/-}$ mice. ApoE$^{-/-}$ mice with the CD45.1 allotype were provided by Dr. Gary Owens (University of Virginia). All mice were either purchased on a pure C57BL/6J background from Jackson Laboratory or backcrossed 10 generations with pure C57BL/6J. Mice were fed a standard chow diet (Tekland 7012) or a Western diet (TD.88137, 42% kcal from fat). Mice were euthanized by CO_2 inhalation. Only male mice were used for experiments. Splenectomy was performed in accordance with ACUC-approved animal protocol by anesthetizing mice with ketamine/xylazine (60-80 mg/5-10kg), excising the spleen, and closing the peritoneal and abdominal wall with absorbable sutures. Sham surgery involved anesthetization, incision, moving spleen with forceps, and suturing the incisions.

Cell preparations for murine and human flow cytometry

During organ harvest, peritoneal cells were harvested by flushing the peritoneal cavity with 10 mL FACS buffer (PBS containing 1% BSA, 0.05% NaN_3). Peripheral blood was collected by cardiac puncture and 20 μL of 0.5 M EDTA was added as an anticoagulant before 50 μL was aliquoted for flow cytometry analysis. Mice were perfused with heparinated PBS then spleen and one femur and tibia were removed. Spleens and flushed bone marrow were filtered through a 70 μm cell strainer. Red blood cells were lysed from single-cell suspensions of bone marrow, spleen and peripheral blood using a lysis buffer containing 155 mM NH_4Cl, 10 mM $KHCO_3$, and 0.1 mM EDTA. Cell surface Fc receptors were blocked using anti-CD16/32 (clone:93,

eBioscience), then cells were stained with fluorescently conjugated antibodies against cell surface markers. Cells were stained with fixable Live/Dead Yellow or Live/Dead Aqua (Life Technologies) for dead cell discrimination, then fixed in 2% PFA in PBS. For B-1a cell sorting, dead cell discrimination was determined using DAPI staining. $DAPI^-$ $CD19^+CD23^-B220^{mid\text{-}lo}IgM^+CD5^+$ B-1a cells were sorted to better than 99% purity from their parent gate. Clone and fluorophore information for the flow cytometry antibodies used in murine experiments to FAC-sort or immunophenotype B cell subsets are given in **Online Table VI**.

Isolation of PBMC's from human peripheral blood was performed as previously described[3] using Ficoll-Paque Plus (GE Healthcare) and SepMate tubes (Stemcell Technologies), and the buffy coat layer was isolated for staining. Clone and fluorophore information for the flow cytometry antibodies used in human flow cytometry experiments to immunophenotype B cell subsets are given in **Online Table VII**.

All flow cytometry analysis was conducted at the University of Virginia Flow Cytometry Core Facility. Immunophenotyping was performed on a CyAn ADP (Beckman Coulter) or an Attune NxT (ThermoFisher) cytometer. Data analysis and flow plots were generated using FlowJo software (Tree Star Inc). Representative flow plots were chosen based on the samples whose population frequencies were closest to the mean for that group. B-1a cell sorting was performed on an Influx or FACS Vantage cell sorter (BD Bioscience). Gates on flow plots were set using fluorescence minus one (FMO) controls.

Single-cell sorting and sequencing of the immunoglobulin heavy chain

$CD45^+CD19^+B220^{mid\text{-}lo}$ $CD43^+CD5^+$ B-1a cells from bone marrow and peritoneal cavity of five 100-week-old chow-fed $ApoE^{-/-}$ mice were single-cell sorted into 96 well plates containing lysis buffer (RNaseOut, 5x Buffer, DTT, IgePAL, carrier RNA, Invitrogen). A 20μl reverse transcription reaction was run per well using the SuperScript III enzyme and random hexamers (Invitrogen). Qiagen's HotStart Taq Plus master mix kit was used to perform the first round of PCR (25μl reaction) using 2.5μl of cDNA diluted 1:2 and the MsVHE and MsCμE primers (**Online Table VIII;**[4]) each at 0.6 μM. Each 25 μl reaction was run as follows: 95°C for 5 minutes; 35 cycles at 94°C for 30 seconds, 50°C for 30 seconds, 72°C for 30 seconds; and then a final extention at 72°C for 10 minutes. The product from this first reaction was then diluted at 1:100 in dH2O and 2 μl was used in the second semi-nested 25 μl reaction using the following MsVHE and MsCμN primers (Supplementary Table 8) each at 0.6 μM. The second reaction was run as follows: 95°C for 5 minutes; 40 cycles at 94°C for 30 seconds, 53°C for 30 seconds, 72°C for 30 seconds; and then a final extention at 72°C for 10 minutes. DNA concentration was determined using the Qiagen Qiaxcel. PCR products were sequenced (Genewiz) using the MsV_HE primer. Sequences were analyzed using an online sequence analysis tool, IMGT/HighV-Quest[5], and only sequences with ≥90% homology to VDJ regions were included.

MDA-LDL generation

MDA-LDL was either provided by Dr. Sotirios Tsimikas (UCSD), or prepared as follows. MDA was freshly generated by incubating malonaldehyde bis dimethylacetal with 1N HCl at 37°C, then the solution was adjusted to pH 7.4 with NaOH. MDA-LDL was prepared by incubating the prepared MDA solution with human LDL (Kalen Biomedical, LLC) at a constant ratio of 100 uL MDA per 1 mg of LDL, for 3 hours at 37°C. After conjugation, MDA-LDL was extensively dialyzed against PBS at 4°C in 10K (10,000 MWCO) Slide-A-Lyzer dialysis cassettes (Thermo Fisher Scientific) to remove excess MDA. Protein concentration was determined by Bradford assay. MDA-LDL was used to coat ELISPOT plates for quantification of anti-MDA-LDL IgM ASC in tissue, as described below.

ELISPOT

Single-cell suspensions of bone marrow and spleen were prepared as described above. ELISPOT to measure total IgM ASC was performed as described previously[3]. Briefly, 10 μg/mL unlabeled anti-mouse IgM antibody (Southern Biotech, 1020-01) or MDA-LDL (prepared as described above) was used to coat Sterile Multiscreen IP-Plates (MSIPS4510). ELISPOT wells were blocked with RPMI1640 containing 10% FCS, then 1-2.5x10^5 spleen or bone marrow cells were added to leading wells and serial 5-fold dilutions of samples were performed. Non-binding cells were washed off with PBS containing 0.01% Tween-20, then ELISPOT wells were incubated in 2 μg/mL biotin-labeled anti-mouse IgM secondary antibody (Southern Biotech, 1020-08) in PBS/0.01%

Tween-20. For detection, sequential incubations with streptavidin alkaline phosphatase (Abcam), then BCIP/NBT were performed. Wells were imaged using a Bioreader 4000 (Biosys) and spots were counted manually. Overall IgM ASC number was back-calculated based on sample dilution or total cell count.

ELISA for quantification of total and anti-OSE IgM in mice and humans

Total IgM, IgG1, IgG2b, IgG2c, and IgG3 in mouse serum or plasma was measured using colorimetric ELISA as follows. EIA/RIA high-binding microplates were coated with goat anti-mouse IgM, IgG1, IgG2b, IgG2c, or IgG3 capture antibody (Southern Biotech, 1020-01; 1070-1; 1090-01; 1079-01; 1100-01). Mouse IgM, IgG1, IgG2b, IgG2c, and IgG3 standards (Southern Biotech, 0101-01; 0102-01; 0104-01; 0122-01; 0105-01), or plasma or serum samples were detected with alkaline phosphatase-conjugated goat anti-mouse IgM, IgG1, IgG2b, IgG2c or IgG3 secondary antibody (Southern Biotech, 1020-04; 1070-04; 1090-04; 1079-04; 1100-04) and pNPP substrate (Southern Biotech 0201-01). Absorbance measurements were analyzed with a SpectraMAX 190 microplate reader (Molecular Devices) at 405 nm. The standard curve was determined using a 4-parameter function and concentration measurements were extrapolated using Softmax Pro 3.1.2 software. Only samples with CV<15% and within the standard curve were included in analysis.

Levels of IgM antibodies specific for MDA-LDL, AB1-2 (anti-idiotypic antibody recognizing E06/T15 specificity), and α-1,3-dextran in mouse serum or plasma were determined by chemiluminescent ELISA as previously described [3, 6, 7]. In brief, microtiter

plates were coated with various antigens at 5 μg/mL in PBS. Antigens used were prepared as previously described [6, 7]. Serially diluted plasma was added, and bound plasma IgM was detected with rat anti-mouse IgM μ-chain conjugated to alkaline phosphatase (Abcam) and LumiPhos 530 solution, using a Dynex Luminometer (Dynex Technologies, Chantilly, VA, USA). Data were expressed as relative light units counted per 100 milliseconds (RLU/100 ms). For each set of mice, plasma pools were made initially and used for formal dilution curves to determine optimal dilution for each antigen to use in binding assays. A specific non-saturating dilution was chosen for each antigen, and then plasma samples from each mouse were assayed to determine mean and SD for that determination.

Levels of IgM or IgG against MDA-LDL in human plasma were measured by chemiluminescent ELISA as previously described[8]. Briefly, 5 ug/mL MDA-LDL was coated on microtiter well plates, plasma was added, and IgG or IgM antibodies binding to MDA-LDL were detected with alkaline phosphatase-conjugated goat anti-human IgM or IgG (Sigma).

In vitro LPS stimulation

Peritoneal B-1a cells from $CXCR4^{WT}ApoE^{-/-}$ and $CXCR4^{BKO}ApoE^{-/-}$ mice were sorted as described above, and cultured *in vitro* in B cell media (RPMI-1640, 10% heat-inactivated fetal bovine serum, 10 mM HEPES, non-essential amino acids, 1 mM sodium pyruvate, 50 ug/mL gentamicin, 55 nM β-mercaptoethanol) containing either 50 μg/mL LPS (Sigma, L4391) or an equivalent volume of PBS. After 72 hours, culture

supernatants were harvested, total IgM was measured by ELISA as described above, and normalized to cell counts plated. The fold change in amount of IgM secreted into supernatants from cells cultured with LPS over cells cultured in PBS from each mouse was compared.

Transmigration assay

Peritoneal lavage cells from $CXCR4^{WT}ApoE^{-/-}$ and $CXCR4^{BKO}ApoE^{-/-}$ mice were isolated and $2.5x10^6$ cells were plated into transwell chambers containing 0.4 micron pores (Costar, Corning Incorporated, 3450). Lower wells contained media either with or without 100 ng/mL CXCL12 to distinguish spontaneous migration from migration towards CXCL12. An input aliquot from each sample was additionally analyzed by flow cytometry. Cells were cultured at 37°C for 6 hours then migrated cells in the lower well were harvested for flow cytometry. Percent specific migration towards CXCL12 was defined as the (# of B cells migrating towards CXCL12 - # of B cells migrating spontaneously)/(# of input B cells loaded in the transwell).

Real-time polymerase chain reaction for analysis of chemokine expression

RNA was extracted from CD19+ splenocytes or total bone marrow cells using the RNeasy Plus mini kit (Qiagen, 74134). 1μg of RNA was then treated with DNase (Invitrogen) and used to reverse transcribe cDNA using an iScript cDNA synthesis kit (BioRad). To quantify gene expression, cDNA was diluted 1:10 in water and combined with 0.5mM forward and reverse primers and SYBR Green (SensiFast, BioLine). Semi-

quantitative real-time PCR was performed on a CFX96 Real-Time System with an annealing temperature of 60°C for all reactions (BioRad). Data were calculated by the ΔΔCt method and expressed in arbitrary units that were normalized to 18s ribosomal RNA levels. Primer sequences are listed in **Online Table VIII**.

Retrovirus production

CXCR4-GFP and Ctl-GFP retroviruses were generated using the pMigR1 retroviral vector (provided by Dr. Timothy Bender, UVA), which expresses GFP. The mouse CXCR4 gene was subcloned from a pCMV-SPORT6 vector (cDNA clone IMAGE:2864967, provided by Dr. Timothy Bender) into the MigR1 retroviral vector using XhoI and EcoRI restriction sites to generate a CXCR4-GFP retroviral vector. The MigR1 (Ctl-GFP) or CXCR4-GFP retroviral vector was co-transfected with the pCL-Eco retroviral packaging vector into 293T cells using calcium phosphate transfection. Transfected 293T cells were incubated at 37°C in an incubator containing 5% CO_2 and viral supernatant was collected 48 hours later. Viral titer was determined by quantifying the frequency of GFP+ cells 24 hours after transduction of 3T3 cells.

Retroviral overexpression of CXCR4 on mouse B cells

Mouse peritoneal B cells were enriched using MACS depletion. Briefly, peritoneal lavage cells from CD45.1^{+} ApoE$^{-/-}$ mice were stained with biotinylated anti-mouse Ter119, CD3e, Gr-1, CD23, NK1.1, and F4/80 antibodies (details in **Online Table IX**), secondarily stained with anti-biotin magnetic beads (Miltenyi Biotec), then depleted over

Miltenyi MACS LS columns. Enriched B cells were cultured overnight in the presence of 100 nM TLR9 agonist CpG ODN 1668 (Invivogen) to stimulate cell proliferation required for retroviral transduction. Twenty-four hours after stimulation, cells were transduced at a 20:1 multiplicity of infection with Ctl-GFP or CXCR4-GFP retroviral particles using spinfection at 2000 rpm for 90 minutes and cultured for 3 hours at 37°C in the presence of polybrene. Then excess virus was washed off and cells were cultured in fresh B cell media (described above) overnight at 37°C. Cells were FACS-sorted for GFP+ B-1a cells to be used for adoptive transfer. Approximately 30-40% of B-1a cells were successfully transduced by this method.

Adoptive Transfer

Following fluorescence activated cell sorting as described above, B-1 cells were adoptively transferred accordingly:

For short-term transfer of CD45.2^{+} ApoE$^{-/-}$ B-1 cells: 1x10^{6} ApoE$^{-/-}$ peritoneal B-1 cells from CD45.2 allotype mice or an equivalent volume control PBS were intravenously injected into CD45.1 allotype ApoE$^{-/-}$ mice. 4 days later, tissues were harvested for flow cytometry.

For short-term transfer of CXCR4-WT and CXCR4-BKO B-1 cells: 750,000 CXCR4^{WT} ApoE$^{-/-}$ or CXCR4^{BKO} ApoE$^{-/-}$ B-1 cells or an equivalent volume PBS were intravenously injected into Rag1$^{-/-}$ ApoE$^{-/-}$ mice via retro-orbital injection. Three hours later, tissues were harvested for flow cytometry.

For transfer of virally transduced B-1a cells: 100,000 Ctl-GFP+ or CXCR4-GFP+ B-1a cells from CD45.1 allotype ApoE$^{-/-}$ mice, or an equivalent volume control PBS, was intravenously injected into CD45.2 allotype Rag1$^{-/-}$ ApoE$^{-/-}$ host mice via retro-orbital injection. Host mice were maintained on chow diet for one week after transfer, then switched to Western diet (TD.88137) for 16 weeks. At the end of diet feeding, tissues were collected for analysis of donor CD45.1+GFP+ B-1a cell localization.

Atherosclerosis analysis

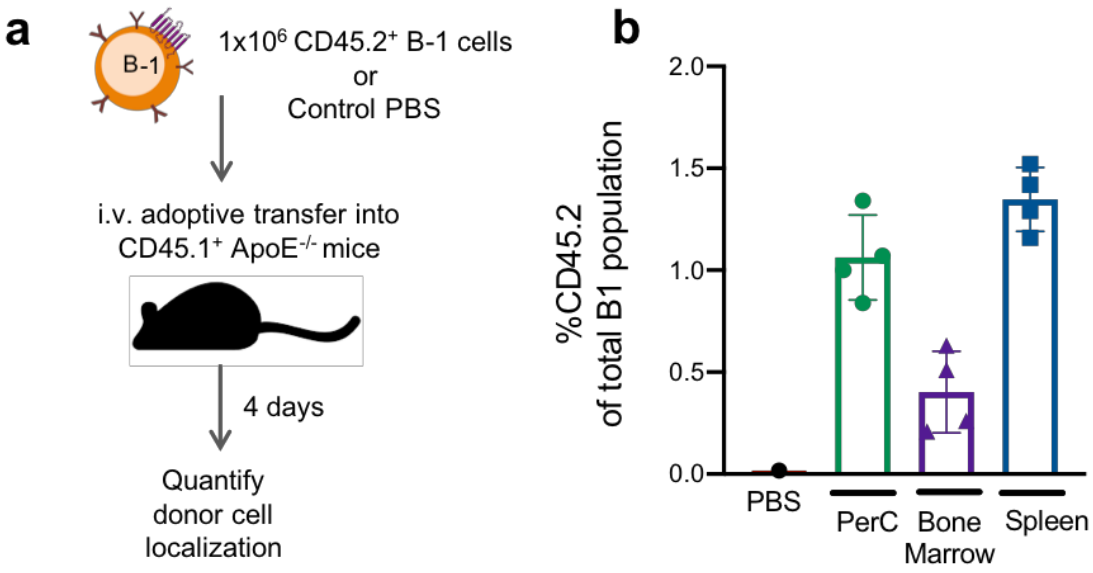
Aortas were perfused with heparinated PBS then opened longitudinally from the aortic arch to the iliac bifurcation, fixed in 4% paraformaldehyde, pinned, and stained using Sudan IV (Sigma) as previously described[2]. Aortas were imaged using a Nikon D70 DSLR camera and the percentage of the total aorta area that was Sudan IV+ plaque area was quantified using Image-Pro Plus software (Media Cybernetics).

Randomization, blinding, power calculation

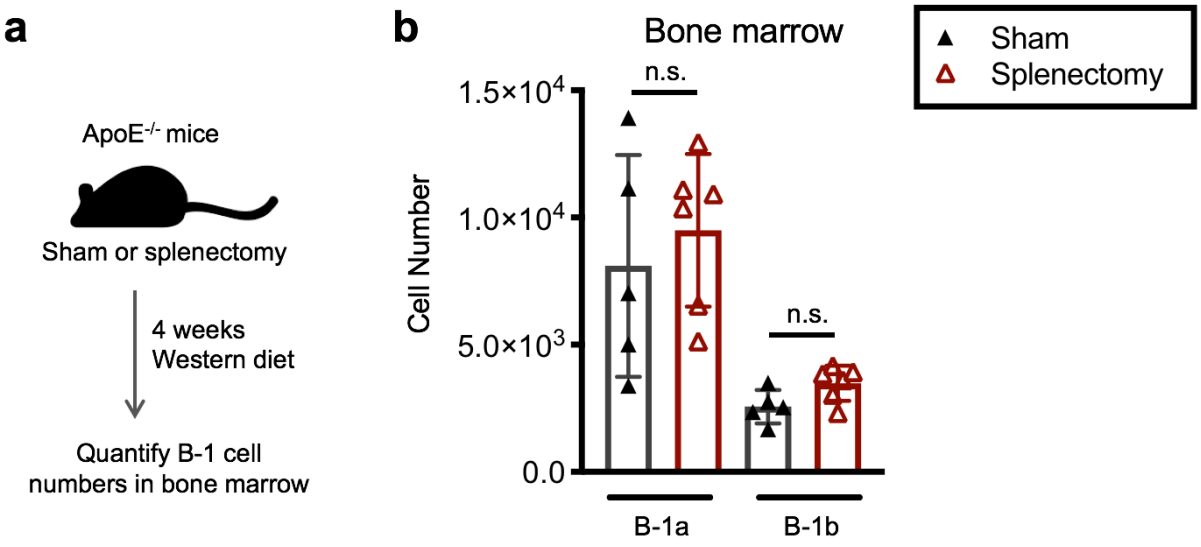
To avoid differences arising from cage to cage variation in mouse experiments, each cage had at least one mouse from each experimental group, but otherwise, experimental groups were allocated randomly, and investigators were blinded to group allocation when performing all data collection. No power calculation was performed in mouse experiments, but the sample size of 5-10 is similar to what has been reported in previous publications.

Statistics

Statistics were performed as indicated using GraphPad Prism Version 7.0a (GraphPad Software, Inc). For comparison of data containing two groups, Mann-Whitney test was performed. Potential single outliers were tested in data sets using two-sided Dixon's outlier test and excluded if $p<0.05$. For comparing more than two groups of data, one-way ANOVA with Tukey's multiple comparisons test was used where data was normally distributed. For comparing more than two groups of non-parametric data, Kruskal-Wallis test was used. χ^2 analysis was performed using 2x4 and 2x2 comparisons as indicated. Results from all replicated experiments are displayed and bar graphs display mean ± standard error of the mean. Correlations were determined using Pearson correlation coefficient (r-value) when both variables passed the D'Agostino and Pearson test for normality. Where one or both variables being correlated failed the D'Agostino and Pearson test for normality, Spearman correlation coefficients are reported. For correlation and in multivariate analysis, log transformation was applied to the MFI of CXCR4 on B1 cells to transform the highly skewed variable into an approximately normal variable. To exclude co-linearity between variables, a Spearman correlation matrix was generated using the PROC CORR procedure in SAS 9.4. Additional co-linearity diagnostics was performed by including tolerance and variance inflation terms in multivariate linear regression modeling using the PROC REG procedure in SAS 9.4. P-values were adjusted for multiple testing by using False Discovery Rate, determined in SAS 9.4.

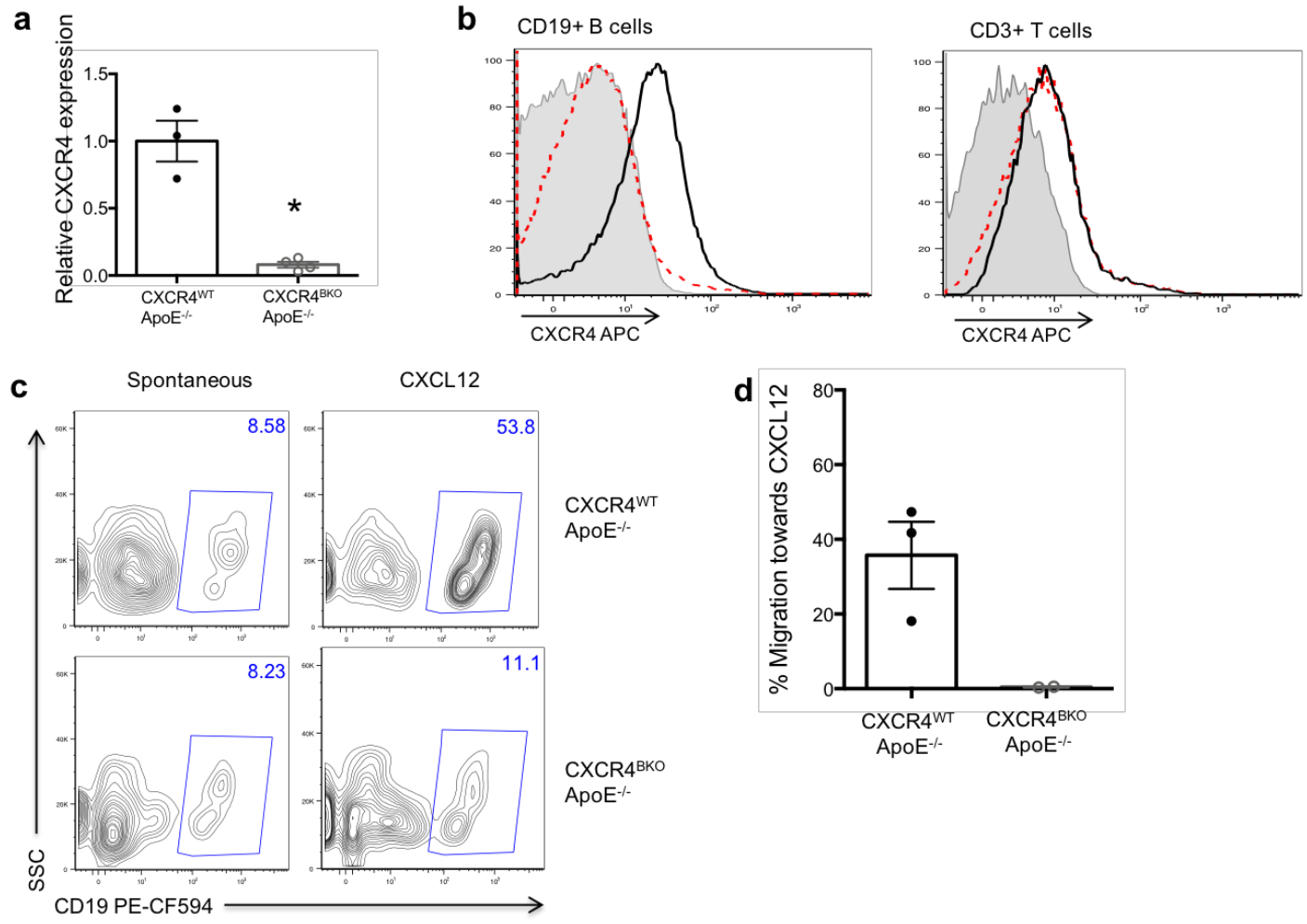


Online Figure I. Mature peritoneal B-1 cells migrate to the bone marrow.
(a) Experimental schematic for short-term 4-day adoptive transfer. (**b**) Calculated frequency of CD45.2^{+} donor B-1 cells recovered from peritoneal cavity (PerC), bone marrow, or spleen of CD45.1 ApoE$^{-/-}$ mice receiving control PBS (n=1) or 1x10^{6} CD45.2^{+} CD19^{+} CD23^{-} peritoneal cavity B-1 cells (n=4).

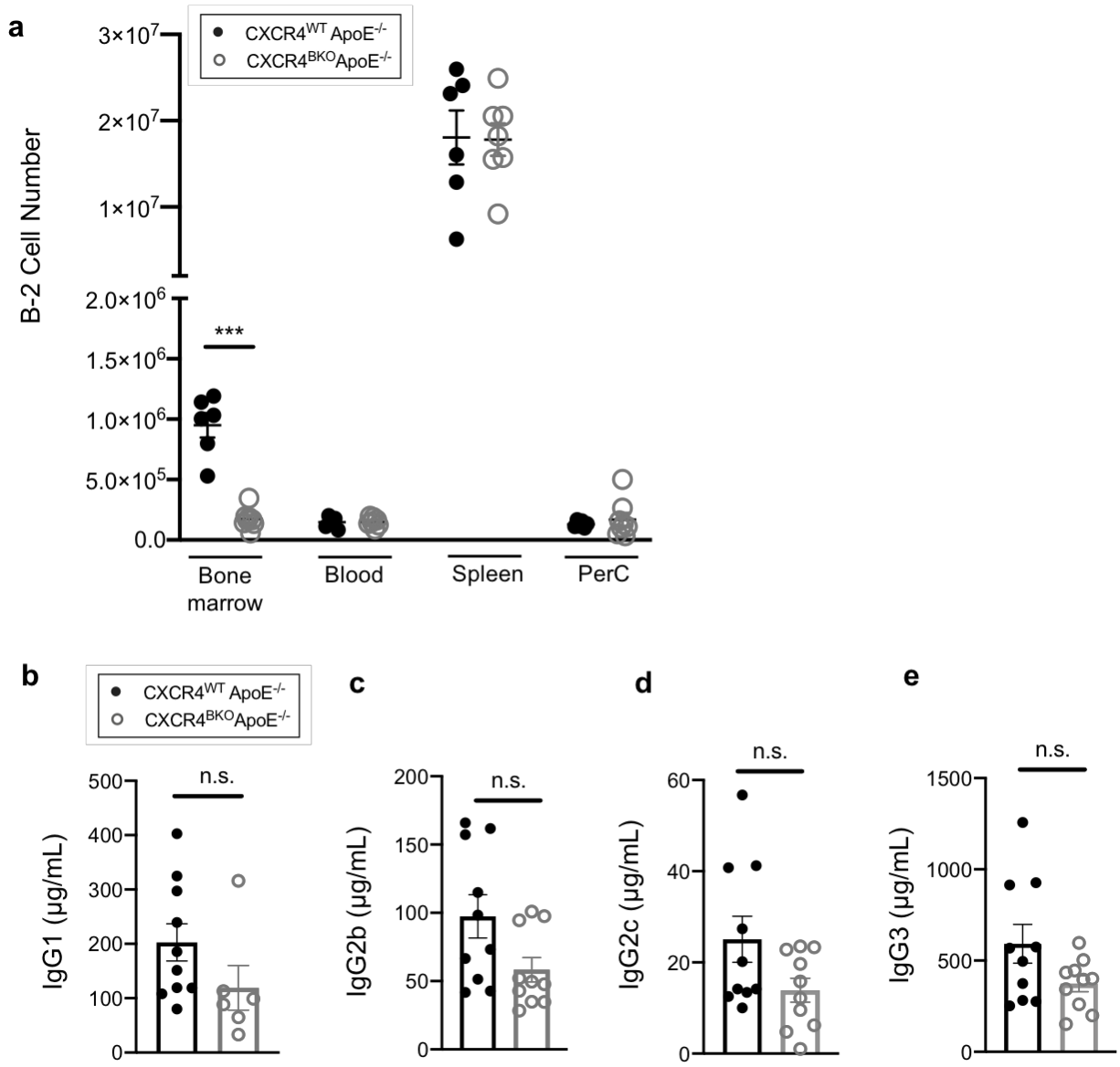


Online Figure II. The adult bone marrow B-1 population does not rely on the spleen. (a) Experimental schematic for splenectomy. (**b**) Calculated number of $CD19^{+}$ $B220^{mid\text{-}lo}$ IgM^{+} $CD23^{-}$ $CD43^{+}$ $CD5^{+\ or\ -}$ B-1a or B-1b cells recovered from bone marrow of $ApoE^{-/-}$ mice receiving splenectomy (n=6) or sham surgery (n=5). N.s. indicates non-significant p-value by Mann-Whitney test.

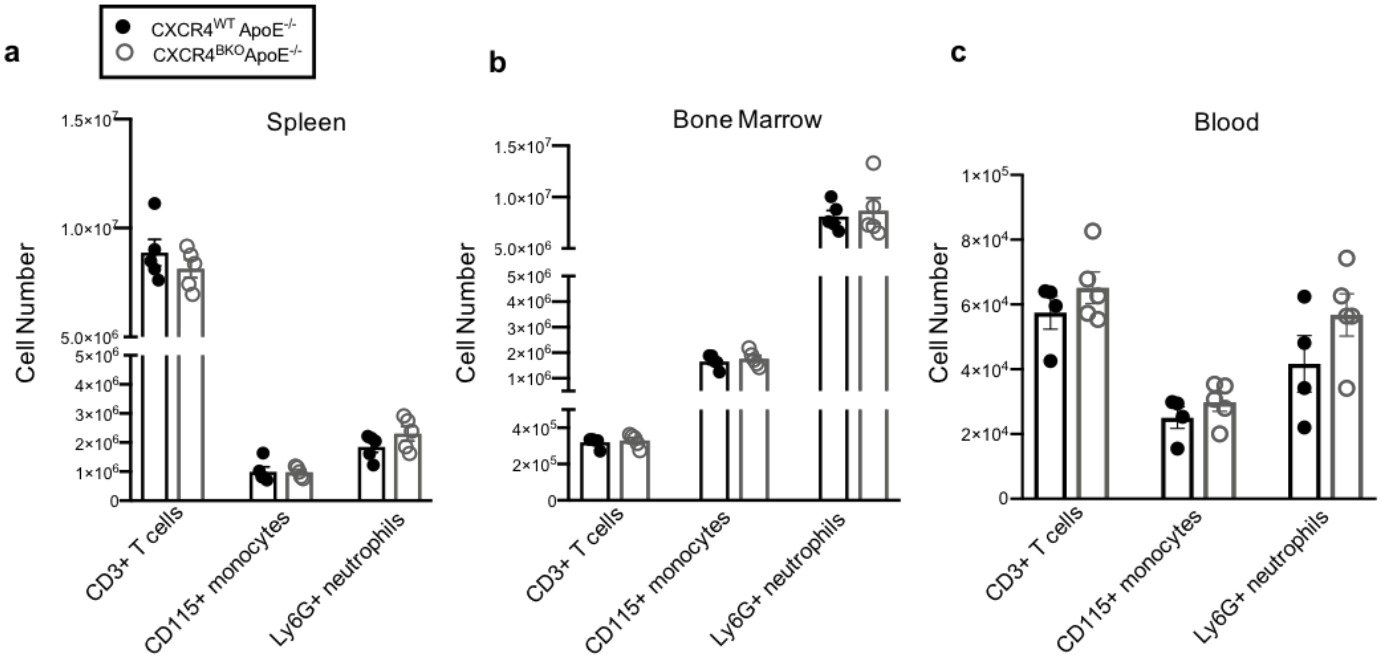
$CXCR4^{WT}$ $ApoE^{-/-}$: $CXCR4^{flox/flox}$ $CD19^{+/+}ApoE^{-/-}$, $CXCR4^{BKO}$ $ApoE^{-/-}$: $CXCR4^{flox/flox}$ $CD19^{cre/+}ApoE^{-/-}$



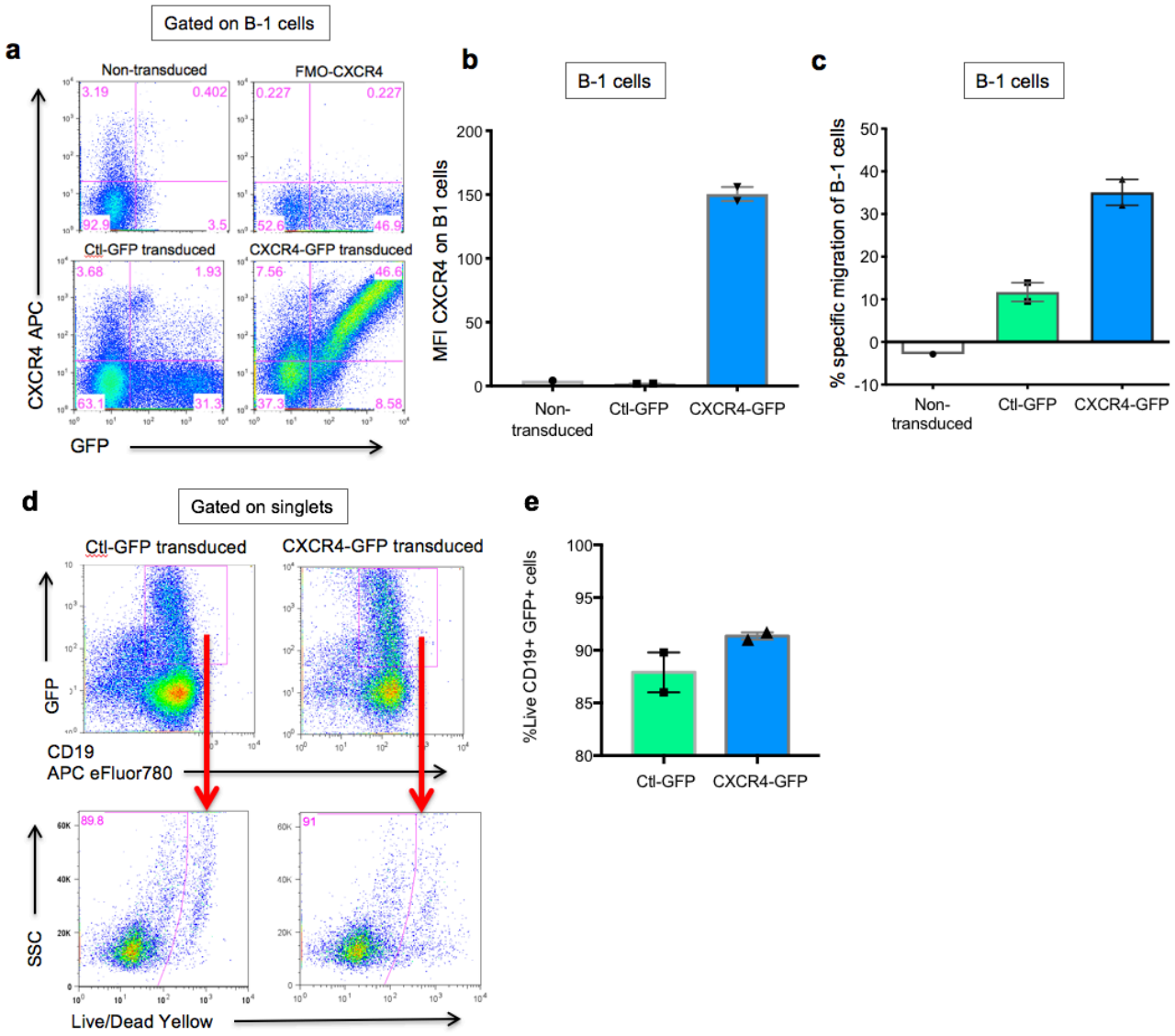
Online Figure III. Generation of a B cell specific knockout of CXCR4 on an atherogenic $ApoE^{-/-}$ background. (**a**) Quantification of CXCR4 mRNA expression in CD19+ splenocytes isolated from $CXCR4^{WT}$ $ApoE^{-/-}$ (n=3) or $CXCR4^{BKO}$ $ApoE^{-/-}$ (n=4) mice. *P<0.05 by two-tailed t-test. (**b**) Surface CXCR4 expression on CD19+ B cells or CD3+ T cells in peripheral blood from $CXCR4^{WT}$ $ApoE^{-/-}$ (solid black histogram) or $CXCR4^{BKO}$ $ApoE^{-/-}$ mice (dashed line histogram). Shaded histograms indicate FMO-CXCR4 control. (**c**) Representative flow plots of the frequency of $CXCR4^{WT}$ $ApoE^{-/-}$ (top) or $CXCR4^{BKO}$ $ApoE^{-/-}$ (bottom) CD19+ cells migrating spontaneously (left) or towards CXCL12 . (**d**) Frequency of B cells isolated from $CXCR4^{WT}$ $ApoE^{-/-}$ (n=3) or $CXCR4^{BKO}$ $ApoE^{-/-}$ (n=2) mice that migrated towards CXCL12 as a percentage of the total number of B cells loaded in transwell. Error bars represent mean ± s.e.m.



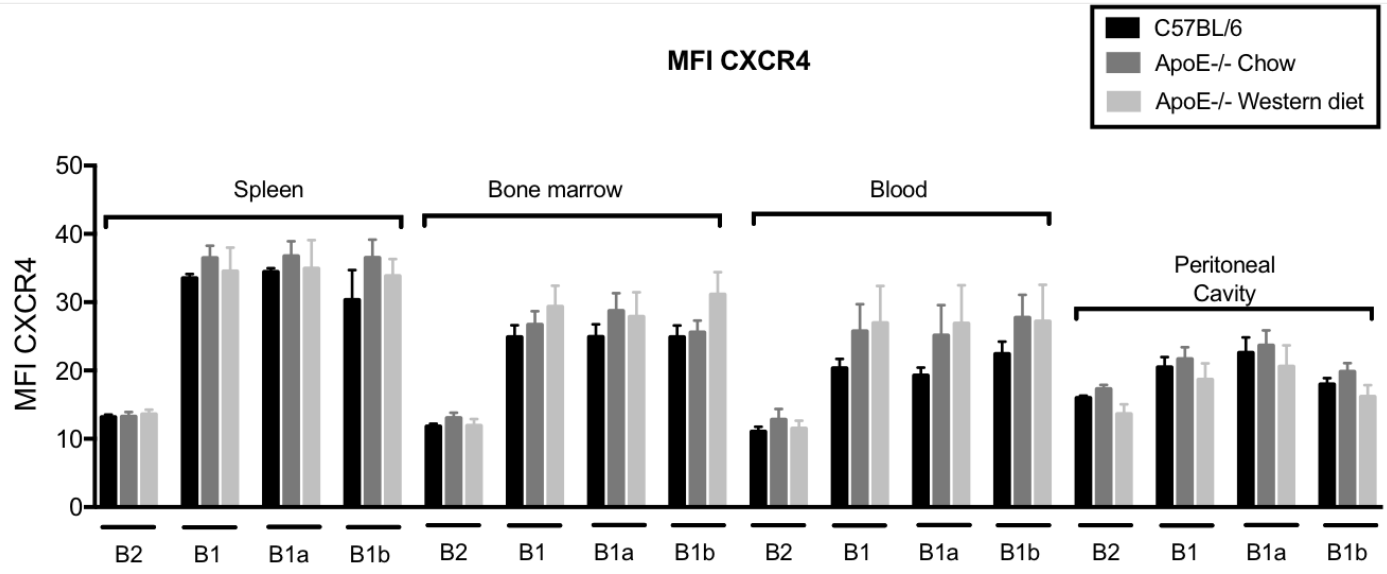
Online Figure IV. B cell-specific loss of CXCR4 reduces mature B-2 number in the BM but does not significantly impair production of IgG antibodies in young ApoE$^{-/-}$ mice. (a) Quantification of CD19^{+}B220^{hi}CD23^{+} B-2 cells from bone marrow, blood, spleen, and peritoneal cavity of 8-week-old CXCR4^{WT} ApoE$^{-/-}$ (n=6) or CXCR4^{BKO} ApoE$^{-/-}$ (n=8) mice. Relative titers of **(b)** IgG1, **(c)** IgG2b, **(d)** IgG2c, and **(e)** IgG3 in sera of 8-week-old CXCR4^{WT} ApoE$^{-/-}$ (n=10) or CXCR4^{BKO} ApoE$^{-/-}$ (n=10) mice. 4 samples excluded in IgG1 dataset due to duplicate measurements having CV>30%. ***P<0.001 or n.s. indicates non-significant p-value using Mann-Whitney test.



Online Figure V. B cell-specific loss of CXCR4 does not significantly impact numbers of other immune cell types in the spleen, bone marrow, or blood. Calculated numbers of CD3+ T cells, CD115+ monocytes, and Ly6G+ neutrophils in spleen (**a**), bone marrow (**b**), or circulating blood (**c**) of 8-week-old CXCR4^{WT} ApoE$^{-/-}$ (n=5) or CXCR4^{BKO} ApoE$^{-/-}$ (n=5) mice. Error bars represent mean ± s.e.m.



Online Figure VI. Retroviral-mediated overexpression of CXCR4 on CXCR4^{BKO} ApoE$^{-/-}$ B cells. Peritoneal B cells from CXCR4^{BKO}ApoE$^{-/-}$ mice were isolated and transduced with Ctl-GFP or CXCR4-GFP retrovirus, or cultured without transduction. (**a**) Representative flow plots of CXCR4 and GFP expression on B-1 cells from non-transduced (upper left), Ctl-GFP transduced (lower left), or CXCR4-GFP transduced (lower right) conditions. FMO-CXCR4 (upper right) used to set CXCR4 positive gate. (**b**) Quantification of the MFI of CXCR4 on GFP+ B-1 cells from non-transduced (n=1), Ctl-GFP transduced (n=2), or CXCR4-GFP transduced (n=2) conditions. *P<0.05 by one-way ANOVA with Tukey's multiple comparisons test. (**c**) Frequency of non-transduced (n=1), Ctl-GFP transduced (n=2), or CXCR4-GFP transduced (n=2) B-1 cells that migrated towards CXCL12 as a percentage of the total number of B-1 cells loaded in transwell. (**d**) Representative gating strategy for quantification of viable cells within the successfully transduced B cell population (CD19+GFP+). (**e**) Frequency of live B cells after transduction with Ctl-GFP (n=2) or CXCR4-GFP retrovirus (n=2). Error bars represent mean ± s.e.m.



Online Figure VII. CXCR4 expression on B cell subsets is not modulated by ApoE knockout or Western diet. Quantification of CXCR4 MFI on splenic, bone marrow, blood, and peritoneal B cell subsets isolated from age-matched male C57BL/6 (n=5), $ApoE^{-/-}$ chow-fed (n=4), and $ApoE^{-/-}$ 8 weeks Western diet-fed (n=4) mice. B-2 cells were identified as $CD19^{+}B220^{hi}CD23^{+}$ cells; B-1 cells were identified as $CD19^{+}$ $B220^{mid\text{-}lo}$ $CD23^{-}$ $CD43^{+}$ IgM^{+} cells, then CD5 was used to discriminate B-1a and B-1b cells. CXCR4 expression was determined using an FMO control. Error bars represent mean ± s.e.m.

Online Table I. Replicate CDR-H3 amino acid sequences (those present more than once) and frequency present in single cell-sorted BM and PerC B-1a cells.

BM B-1a (n=258 total sequences)			PerC B-1a (n=310 total sequences)		
CDR-H3 Sequence	**# of CDR-H3 replicates**	**% of total sequences**	**CDR-H3 Sequence**	**# of CDR-H3 replicates**	**% of total sequences**
AGDYDGYWYFDV	30	11.6	AGDYDGYWYFDV	217	70.0
AREVTTMYYFDY	26	10.1	AGDRWGYWYFDV	13	4.2
ARSYYYGSSYYFDY	21	8.1	ARSYYYGSSYYFDY	13	4.2
AREDYYGSSYYFDY	8	3.1	ARYYGNYWYFDV	6	1.9
AGDRTGYWYFDV	6	2.3	AGDLLGYWYFDV	5	1.6
AIYYGNYWYFDV	5	1.9	ARDYYWYFDV	4	1.3
ATYYSNYWYFDV	5	1.9	AGDSDGYWYFDV	4	1.3
TTYYYGSAWFAY	5	1.6	AGDYYGYWYFDV	3	1.0
AKRDYYSNYFGLGYYYAMDY	5	1.6	ASYYGNYWYFDV	2	0.6
ATGSSFDY	4	1.6	ATYYSNYWYFDV	2	0.6
ASYYGNYWYFDV	4	1.6	AREGDYYYGSSYWFAY	2	0.6
MRYSNYWYFDV	4	1.6	MRYSNYWYFDV	2	0.6
AKNDYGIYYYAMDY	4	1.6	AGDTTGYWYFDV	2	0.6
ARSNYYAMDY	3	1.2	AGDRTGYWYFDV	2	0.6
MRYGDYWYFDV	3	1.2	AGDPYDGYYGFAY	2	0.6
AGDADGYWYFDV	3	1.2	AGDRDGYWYFDV	2	0.6
AGDYYGYWYFDV	3	1.2	AKLR*XLL*L	2	0.6
TPHYYGSSWFAY	3	1.2	ARYYYGSSYAMDY	2	0.6
ARNGGLWSYYAMDY	3	1.2			
ARMGNYGSRYFDV	2	0.8			
AREQLRLYYFDY	2	0.8			
ARRYYYGSSYAMDY	2	0.8			
ANWAY	2	0.8			
ARSSNYAMDY	2	0.8			
ARDYYWYFDV	2	0.8			
MRYGYDWYFDV	2	0.8			
AGDRWGYWYFDV	2	0.8			
AGYAWFAY	2	0.8			
TYYGNYENYTVDY	2	0.8			
AKNNYYGGYFDV	2	0.8			
TRPSTVHFDY	2	0.8			

Online Table II. Associations between circulating levels of anti-MDA-LDL antibodies and CXCR4 expression on other B cell subsets or the frequency of other B cell subsets. Data presented as correlation coefficient (r) and statistical significance (p). N=50 subjects. *P<0.05.

Variable	**MDA-LDL IgM**		**MDA-LDL IgG**	
	r-value	**p-value**	**r-value**	**p-value**
MFI CXCR4 on total CD20+ B cells	0.05	0.73	0.20	0.16
%CD27-CD43- naïve B of total CD20+ B cells	-0.31	0.031*	0.048	0.74
MFI CXCR4 on CD27-CD43- naïve B	0.18	0.22	0.22	0.12
%CD27+CD43- memory B of total CD20+ B cells	0.30	0.031*	-0.059	0.69
MFI CXCR4 on CD27+CD43- memory B	-0.056	0.70	0.11	0.45

Online Table III. Clinical and laboratory characteristics of IVUS subjects. Values are presented as n(%) for binary measures or as mean± SD.

Subject Characteristics	**Values**
Age (y)	58.7 ± 9.6
Gender n (%male) n (%female)	27 (54%), 23 (46%)
Race n (%African Americans) n (%Caucasians)	3 (6%), 47 (94%)
Body Mass Index (kgm^{-2})	31.7 ± 7.0
Hypertension n(%)	41(82%)
Type-2 Diabetes n (%)	20 (40%)
Statin use n (%)	42 (84%)
Hemoglobin (k/µl)	12.7 ± 2.4
Hematocrit (%)	38.2 ± 4.3
Platelets (k/µl)	221 ± 55
White blood cell count (k/µl)	7.2 ± 2.7
Serum Creatinine (mg/dl)	0.9 ± 0.17
eGFR (MDRD) (ml/min per 1.73 m^2)	87.2 ± 14.2
Total cholesterol (mg/dl)	148 ± 35
Triglycerides (mg/dl)	116 ± 71
High density lipoprotein cholesterol (mg/dl)	39.8 ± 11
Low density lipoprotein cholesterol (mg/dl)	89.3 ± 30

Online Table IV. Associations of the frequency of circulating B-1 cells with IVUS virtual histology measurements. Given values represent correlation coefficient (r), and statistical significance (p). N=50 subjects.

Variable	**%CD27+CD43+ B-1 cells of total CD20+ B cells**	
	R-value	**p-value**
Plaque Burden	-0.0073	0.96
Stenosis	-0.042	0.77
%Fibrous	-0.028	0.84
%Necrotic	0.011	0.94
%Calcium	-0.11	0.46

Online Table V. Associations of the MFI of CXCR4 on circulating human B-1 cells with other clinical characteristics. Given values represent correlation coefficient (r), and statistical significance (p). N=50 subjects.

Variable	**MFI CXCR4 on B-1 cells**	
	R-value	**p-value**
hsCRP (mg/L)	0.20	0.16
BMI	0.025	0.86
A1c%	-0.18	0.23
Creatinine (mg/dL)	-0.053	0.71

Online Table VI. Flow cytometry antibody fluorophore, clone, and catalog number information for murine experiments.

Marker	Fluorophore	Clone	Vendor	Catalog Number
CD19	APC-eFluor780 PE-Cy7	eBio1D3	eBioscience	47-0193-82; 25-0193-82
B220	APC, eFluor450, PE-Cy7	RA3-6B2	eBioscience	17-0452-83; 48-0452-82; 25-0452-82
CD5	PE	53-7.3	eBioscience	12-0051-83
CD23	PE-Cy7	B3B4	eBioscience	25-0232-82
IgM	FITC, eFluor450	eB121-15F9	eBioscience	11-5890-82; 48-5890-82
CXCR4	APC	2B11	eBioscience	17-9991-82
CD3	eFluor450	eBio500A2	eBioscience	48-0033-82
CD115	PE	AF598	eBioscience	12-1152-81
Streptavidin	APC-eFluor780	-	eBioscience	47-4317-82
CD43	FITC, Biotin	S7	BD Biosciences	553270; 553269
CD19	PE-CF594	1D3	BD Biosciences	562291
IgM	PE-CF594	R6-60.2	BD Biosciences	562565
CD45.1	PerCP-Cy5.5	A20	BD Biosciences	560580
CD45.2	BV421	104	BD Biosciences	562895
Ly6G	BV711	1A8	Biolegend	127643

Online Table VII. Flow cytometry antibody fluorophore, clone, and catalog number information for human flow cytometry experiments.

Marker	Fluorophore	Clone	Company	Catalog Number
CD3	PE-CF594	UCHT1	BD Biosciences	562280
CD20	APC-H7	2H7	BD Biosciences	560734
CD27	BV421, FITC	M-T271	BD Biosciences	562513; 555440
CD43	FITC, BV421	1G10	BD Biosciences	555475; 562916
CD184	APC	2B11	eBioscience	17-9991-82

Online Table VIII. Primer sequences for semi-quantitative real-time PCR analysis of gene expression in murine spleen, bone marrow, or peritoneal cavity cells.

Primer	**Sequence**
Mouse CXCR4 Forward	5'- TAT TGT CCA CGC CAC CAA -3'
Mouse CXCR4 Reverse	5'- CTT TTC AGC CAG CAG TTT CC-3'
Mouse CXCL12 Forward	5'- GGA CGC CAA GGT CGT CGC CGT G -3'
Mouse CXCL12 Reverse	5'- TTG CAT CTC CCA CGG ATG TCA G -3'
Mouse MIF Forward	5'- TGC CCA GAA CCG CAA CTA CAG TAA -3'
Mouse MIF Reverse	5'- TCG CTA CCG GTG GAT AAA CAC AGA -3'
Mouse CXCL13 Forward	5'- TCC TGG GAA GCT GGT GCA ATG -3'
Mouse CXCL13 Reverse	5'- TCA TCA GGG TCA CAG TGC AAA GG -3'
MsV_HE	5' - GGG AAT TCG AGG TGC AGC TGC AGG AGT CTG G - 3'
MsCμE	5' - ATG GCC ACC GAA TTC TTA TCA GA – 3'
MsCμN	5' - TGT AAA ACG ACG GCC AGT CAT TTG GGA AGG ACT GA - 3'

Online Table IX. Flow cytometry antibody clone, and catalog number information for biotinylated antibodies used in magnetic activated cell sorting (MACS) purification of peritoneal cavity B cells for retroviral transduction.

Marker	Conjugated with:	Clone	Company	Catalog Number
Ter119	Biotin	Ter119	eBioscience	13-5921-82
CD3e	Biotin	eBio500A2	eBioscience	13-0033-85
Gr-1	Biotin	RB6-8C5	eBioscience	13-5931-82
CD23	Biotin	B3B4	eBioscience	13-0232-81
NK1.1	Biotin	PK136	BD Biosciences	553163
F4/80	Biotin	BM8	Life Technologies	MF48015

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