SUPPLEMENTAL MATERIAL

Overarching aims and description of the study. *Aim 1:* To quantify IRA B cells in two mouse models of atherosclerosis and in human atherosclerosis. These data are shown in Figure 1 and Supplemental Figure 1. *Aim 2*: To determine whether IRA B cells are important in mouse atherosclerosis and to profile key features such as lesion size, cell accumulation, etc. To achieve this, mixed chimeric mice were generated (described below). These data are shown in Figure 2 and Supplemental Figures 2 and 3. *Aim 3*: To profile the observed differences in T cells more rigorously. These data are shown in Figure 3 and Supplemental Figure 4. *Aim 4*: To link IRA B cells with the observed differences mechanistically. These data are shown in Figure 4 and Supplemental Figure 5 describing the interaction between IRA B cells and Dendritic cells. *Aim 5*: To rescue the phenotype and thus link IRA B cells to the phenotype functionally. These data are shown in Figure 5. Overall, the study used 235 mice. After generation of mixed chimeric mice, the blood leukocyte profile was analyzed and only animals that were reconstituted successfully were placed in the study. Typically ~93% of mixed chimeras were reconstituted and used in the study. The number of mice used in each experiment is indicated in the Figure legends. All mice admitted to the study survived the intended duration of the study.

Supplemental Methods

Animal models and in vivo interventions. *Mixed bone marrow chimeras:* 8 weeks old male *Ldlr*$^{-/-}$ mice were lethally irradiated (950cGy) and reconstituted with a 50:50 mixture of *Csf2*$^{-/-}$ with WT (Controls) and μMT bone marrow cells (IRA B KO), respectively. In analogy male *Ldlr*$^{-/-}$ were reconstituted with a 50:50 mixture of CD45.1^{+} and *Myd88*$^{-/-}$ or *Csf2rb*$^{-/-}$ bone marrow. To induce atherosclerosis *Ldlr*$^{-/-}$ mice consumed a high-fat/cholesterol diet (HCD; D12108C, Research Diets, New Brunswick, NJ, USA) and *Apoe*$^{-/-}$ were placed on a Western diet (TD.88137, Harlan Teklad, Indianapolis, IN, USA) at 8 weeks of age for different time periods as indicated in the text. Bone marrow chimeras were switched to HCD 6 weeks after reconstitution. *Adoptive transfer:* B cells from WT and *Csf2*$^{-/-}$ mice were isolated by magnetic cell separation. Pooled cell suspensions from spleens and peritoneal lavages were incubated with 4 μl anti-CD19-PE Ab (Biolegend, San Diego, CA, USA) per 1 x 10^8 cells in sterile 2% FBS (fetal bovine serum, Atlanta Biologicals, Lawrenceville, GA, USA), 0.5% BSA (bovine serum albumin, MP Biomedicals, Solon, OH, USA) in PBS for 30 min on ice, washed and incubated with 100 μl anti-PE MACS beads (Miltenyi Biotec, Auburn, CA, USA) per 1 x 10^8 cells in 0.5% BSA, 2mM EDTA in PBS for another 30 min on ice. Labeled cells were positively selected in a Midi MACS separator and LS column according to the manufacturer's instructions. Cells were manually counted in a Neubauer chamber, a purity of 93% and viability of over 95% were confirmed by flow cytometric analysis and Trypan blue staining, respectively. At 6 weeks after reconstitution IRA B KO mice received either 25 x 10^6 B cells/mouse from WT or *Csf2*$^{-/-}$ mice by tail vein injection. Mice were placed on HCD and received another 25 x 10^6 B cells/mouse after 4 weeks. Mice were sacrificed after 8 weeks on HCD. When 25 x 10^6 CD45.1^{+} CD19^{+} cells were transferred, twice, 4 weeks apart into CD45.2^{+} IRA B KO mice, 66,542 ± 24,556 CD45.1^{+} IRA B cells were still retrieved after 8 weeks (n = 3, mean ± SEM).

Cell isolation. Peripheral blood was collected by retroorbital bleeding with heparinized capillaries, and erythrocytes were lysed in RBC Lysis buffer (Biolegend). Peritoneal lavages and organs were harvested at day of sacrifice. Spleens, femurs, aortas and paraaortic lymph nodes (1 proximal, 1 abdominal, 2 distal lymph nodes at aortic bifurcation per mouse) were excised after vascular perfusion with 10 ml sterile PBS. Minced spleens and flushed bone marrow were strained through a 40 μm-nylon mesh (BD Biosciences, San Jose, CA, USA). Spleen cell suspensions were further subjected to RBC lysis. Aortas were minced and digested in 450 U/ml collagenase I, 125 U/ml collagenase XI, 60 U/ml DNase I and 60 U/ml hyaluronidase (Sigma-Aldrich, St. Louis, MO, USA) PBS for 1 h at 37°C while shaking. Lymph nodes were minced and digested in 168 U/ml collagenase III (Worthington Biochemicals, Lakewood, NJ, USA), 60 U/ml DNase I (Sigma-Aldrich), 2% FBS RPMI-1640 (Cellgro, Manassas, VA, USA) for 1h at 37°C while shaking. Cells were counted in a Neubauer chamber. One femur contains ~5% of all bone marrow cells [1]. Bone marrow cell counts were extrapolated accordingly. *Cell sorting:* IRA B cells were expanded in vivo by 4 daily intraperitoneal injections of 25mg LPS per mouse or by 3 months of HCD in

Ldlr$^{-/-}$ mice. Spleens from LPS stimulated WT and *Csf2*$^{-/-}$ mice and from atherosclerotic *Ldlr*$^{-/-}$ mice were homogenized and incubated with 4 µl anti-CD138-Biotin Ab (clone 281-2, BD Biosciences) per 1 x 10^8 cells in sterile 2% FBS, 0.5% BSA in PBS for 30 min on ice, followed by incubation with 100 µl anti-Biotin MACS beads (Miltenyi Biotec) per 1 x 10^8 cells in 0.5% BSA, 2mM EDTA in PBS for another 30 min on ice. Cells were positively selected with a Midi MACS separator and LS column and stained for anti-IgM-Fitc (BD Biosciences), anti-CD45.2-PerCp-Cy5.5 (BD Biosciences), anti-CD23-PECy7 (Biolegend), anti-CD43-APC (BD Biosciences), Streptavidin-Alexa Fluor 700 (Life Technologies, Carlsbad, CA, USA), anti-CD19-APCCy7 (Biolegend). CD45.2$^+$, CD23^{low}, IgM^{high}, CD43$^+$, CD138^{high} cells were sorted on a FACS Aria II cell sorter (BD Biosciences). Splenocytes from OT-II mice were stained with anti-CD45.2-Fitc (BD Biosciences), anti-CD4-PE (BD Biosciences), anti-CD25-APC (BD Biosciences), and transgenic CD4$^+$ CD25$^-$ T cells were sorted on a FACS Aria II cell sorter. Splenocytes from IRA B KO and controls and from CD45.1$^+$ C57Bl/6J mice were incubated with 4 µl anti-CD19-Biotin Ab (BD Biosciences) and anti-CD11b-Biotin (BD Biosciences) per 1 x 10^8 cells in sterile 2% FBS, 0.5% BSA in PBS for 30 min on ice, followed by incubation with 100 µl anti-Biotin MACS beads (Miltenyi Biotec) per 1 x 10^8 cells in 0.5% BSA, 2mM EDTA in PBS for another 30 min on ice. Cells were negatively selected with a Midi MACS separator and LS column enriching for T cells and stained for anti-CD45.2-Fitc (BD Biosciences), anti-CD4-PE (BD Biosciences), anti-CD25-APC (BD Biosciences), anti-CD45.1-Alexa700 (Biolegend). Regulatory T cells (T_{reg}) from IRA B KO and control mice were sorted as CD45.2$^+$ CD4$^+$ CD25$^+$ cells, while conventional T cells (T_{conv}) were sorted as CD45.1$^+$ CD4$^+$ CD25$^-$ cells on a FACS Aria II cell sorter (BD Biosciences). TCRβ$^-$ B220$^-$ MHCII^{high}, CD11c^{high} cDC were sorted from splenocytes directly into RLT buffer for subsequent RNA isolation.

Serum analysis. *Cholesterol measurement:* Serum was collected after overnight fasting. Total cholesterol levels were measured with the Cholesterol E colorimetric assay (Wako Chemicals, Richmond, VA, USA) in a Safire^2 microplate reader (Tecan, Maennedorf, Switzerland) according to the manufacturer's instructions. VLDL, LDL and HDL cholesterol were determined by Skylight Biotech (Skylight Biotech, Inc., Japan) *Immunoglobulin (Ig) measurement:* Total serum IgG and IgM were measured by ELISA (Bethyl Laboratories, Montgomery, TX, USA) according to the manufacturer's instructions. Isotype- and antigen-specific antibody titers were determined by chemiluminescent enzyme immunoassays as previously described [2]. In brief, antigens were coated at 5 µg/mL PBS overnight at 4°C (IgG and IgG_{2c} (goat anti-ms-IgG (Pierce 31160)), IgG_1 (Rat anti-ms-IgG1 (BD 553445)), AB-12, CuOxLDL, MDA-LDL). The plates were blocked with 1% BSA in TBS, serially diluted antisera from individual mice were added, and the plates incubated for 1.5h at room temperature. Bound plasma immunoglobulin (Ig) isotype levels were detected with various anti-mouse Ig isotype-specific alkaline phosphatase (AP) conjugates (Abcam) using LumiPhos 530 (Lumigen, Southfield, MI, USA) solution, and a Dynex Luminometer (Dynex Technologies, Chantilly, VA, USA). Data are expressed as relative light units counted per 100 milliseconds (RLU/100 ms). *GM-CSF ELISA:* GM-CSF was measured in undiluted serum with the Mouse GM-CSF Quantikine ELISA Kit (assay range 7.8-500pg/ml) according to the manufacturer's instructions.

Histology. Spleens were embedded in Tissue-Tek O.C.T compound (Sakura Finetek, Torrance, CA, USA), frozen in ice-cold 2-Methylbutane (Fisher Scientific, Fair Lawn, NJ, USA) and sectioned into 6 µm slices yielding 30-40 sections per mouse. The following antibodies were used for immunofluorescence staining: Anti-GM-CSF (clone MP1-31G6; Abcam, Cambridge, MA, USA), secondary biotinylated anti-rat IgG (Vector Laboratories, Burlingame, CA, USA), streptavidin-Alexa Flour 594 (Life technologies), and FITC anti-IgM (clone II/41; BD Biosciences). Images were recorded using a BX63 motorized microscope (Olympus, Center Valley, PA, USA). Murine aortic roots were embedded in Tissue-Tek O.C.T compound, frozen and sectioned into 5 µm slices yielding 30-40 sections per root. Sections that capture the maximum lesion area were used to compare lesion sizes between study groups. Adjacent sections were used for additional immunohistochemical staining. Following stains were performed to assess lesions size and composition: Hematoxylin and eosin (H&E), Oil-Red-O (ORO; Sigma-Aldrich) for lipids, anti-Mac3 (clone M3/84; BD Biosciences) for macrophages, anti-α-smooth muscle actin (ab5694; Abcam) for smooth muscle cells (SMA), Masson's Trichrome staining for collagen (Masson), anti-CD4 (clone RM4-5; BD Biosciences) for T helper cells. Biotinylated secondary antibodies

and avidin-complex were used, and all sections counterstained with hematoxylin. Images were recorded using a Nanozoomer 2.0RS (Hamamatsu Photonics, Hamamatsu City, Japan). En-face ORO staining was performed on pinned aortas after fixation in 10% formalin. Human spleen samples were obtained from surgical specimens and autopsy at the Department of Pathology, Toronto General Hospital, Toronto, ON, Canada. All immunohistochemistry studies on human patients samples were approved by the research ethics board at University Health Network. Spleens from 4 patients without a history or signs of cardiovascular disease upon examination were compared to those from 4 patients with symptomatic cardiovascular disease. Samples were fixed in 10% formalin and embedded in paraffin for histologic sectioning (4 μm thick slices). Following dewaxing and heat-induced antigen retrieval sections were blocked with donkey serum for 10min and stained with primary antibodies rabbit anti-human GM-CSF (bs-3790R, Bioss Inc. MA, USA) and goat anti-human IgM (NB7436, Novus Biologicals, CO, USA) over night. Donkey anti-rabbit Cy3 and donkey anti-goat Cy5 were used as secondary antibodies (Millipore, Billerica, MA, USA) for immunohistochemical staining. Images were recorded with an Olympus Fluo View 1000 confocal laser scanning microscope (Olympus, Tokoyo, Japan).

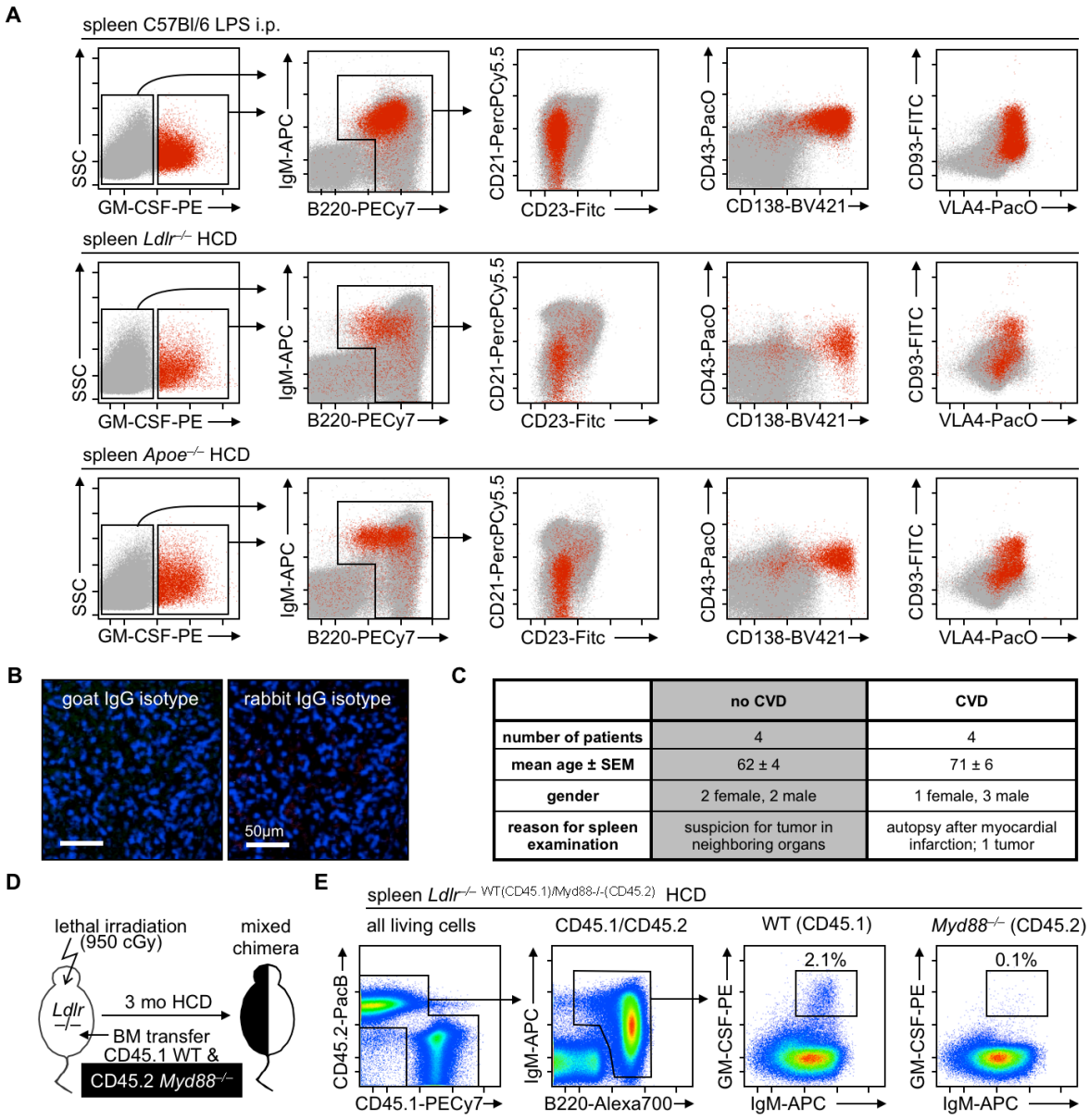
Flow Cytometry. Cell suspensions were stained in PBS supplemented with sterile 2% FBS and 0.5% BSA. The following monoclonal antibodies were used for flow cytometric analysis: anti-Ly6C (clone AL-21, BD Biosciences), anti-CD34 (clone RAM34, BD Biosciences), anti-IgM (clone II/41, BD Biosciences), anti-CD45.1 (clone A20, Biolegend), anti-CD45.2 (clone 104, BD Biosciences), anti-CD86 (clone GL1, BD Biosciences), anti-CD3e (clone 145-2C11, ebioscience), anti-CD4 (clone GK1.5, Biolegend) , anti-CD8 (clone 53-6.7, BD Biosciences), anti-CD90.2 (clone 53-2.1, BD Biosciences), anti-TCRβ (clone H57-597, Biolegend), anti-CD19 (clone 6D5, Biolegend), anti-B220 (clone RA3-6B2, BD Biosciences), anti-CD25 (clone PC61, BD Biosciences), anti-MHCII (clone AF6-120.1, BD Biosciences), anti-F4/80 (clone BM8, Biolegend), anti-CD49b (clone DX5, BD Biosciences), anti-NK1.1 (clone PK136, BD Biosciences), anti-Ly6G (clone 1A8, BD Biosciences), anti-Gr-1 (clone RB6-8C5, ebioscience), anti-CD11b (clone M1/70, BD Biosciences), anti-CD11c (clone HL3, BD Biosciences), anti-CD115 (clone AFS98, ebioscience), anti-CD21 (clone 7E6, Biolegend), anti-CD23 (clone B3B4, Biolegend), anti-CD40 (clone 3/23, Biolegend), anti-CD43 (clone S7, BD Biosciences), anti-CD93(clone AA4.1, BD Biosciences), anti-CD49d/VLA4(R1-2, BD Biosciences), anti-CD44 (clone IM7, Biolegend), anti-CD117/ckit (clone 2B8, BD Biosciences), anti-Ly6A/Sca1 (clone D7, ebioscience) , anti-CD127/ILRα (clone A7R34, ebioscience), anti-CD138 (clone 281-2, BD Biosciences), anti-CD103 (clone 2E7, Biolegend), anti-Ter119 (clone Ter-119, BD Biosciences), anti-GM-CSF (MP1-22E9, BD Biosciences), anti-Foxp3 (clone FJK-16s, ebioscience), anti-IFNγ (clone XMG1.2, BD Biosciences). For intracellular staining cells were fixed and permeabilized with BD Cytofix/Cytoperm (BD Biosciences) according to the manufacturer's instructions. For intracellular staining of IFNγ, IL-4, and IL-17, cell suspensions were stimulated in 2% FBS RPMI-1640 medium with 20 ng/ml PMA and 1 μg/ml ionomycin (Sigma-Aldrich) in the presence of GolgiStop and GolgiPlug (BD Biosciences) for 3 hours at 37°C, 5% CO_2 prior to fixation and permeabilization. For proliferation assays, target cells were labeled with Cell Tracer Violet (Life Technologies) and live cells were identified with Fixable Viability Dye eFluor 780 (ebioscience). Data were acquired on a LSRII and analyzed with FlowJo (Tree Star, Ashland, OR, USA). Specifically, monocytes were identified as $CD45^+$, Lin_1^- (Lin_1 = Ter119, CD3, CD90.2, CD19, B220, NK1.1, CD49b, Ly6G), $CD11b^+$, $MHCII^{low}$, $CD11c^{low}$, $CD115^+$ cells, subdivided into $Ly6C^{high}$ and $Ly6C^{low}$ cells. Neutrophils were identified as $CD45^+$, Lin_1^+, $CD11b^+$, $MHCII^{low}$, $CD11c^{low}$, SSC^{high}, $Ly6C^{int}$ cells. Unless otherwise noted, B cells were identified as $CD45^+$, $CD19^+$ cells. $CD4^+$ and $CD8^+$ T cells were identified within the $CD45^+$, $CD19^-$ $CD3^+$ population. Classical Dendritic cells (DC) were identified as $CD45^+$, $CD19^-$, $MHCII^{high}$, $CD11c^{high}$ cells. PreDC were identified as $CD45^+$, Lin_1^-, F4/80$^-$, CD11b$^-$, CD115$^-$, $MHCII^{low}$, $CD11c^+$ cells. Lin_2^- (Lin_2 = Ter119, CD3, CD90.2, CD19, B220, NK1.1, CD49b, Gr-1, CD11b, CD11c, IL7Rα), $ckit^+$, Sca1$^-$, $CD34^{high}$, $CD16/32^{high}$ myeloid progenitors were identified as GMP when CD115$^-$ and as $ckit^{high}$ MDP and $ckit^{low}$ CDP when $CD115^+$.

Reverse transcription PCR. *Cells:* 1 x 10^5 sorted $CD3^+$, $CD19^+$, $CD11b^+$ splenic cells were lysed in RLT buffer with 1% β-mercaptoethanol. RNA was isolated with the RNeasy Micro Kit (Qiagen, Venlo, Netherlands) followed by cDNA transcription with the iScript Select cDNA Synthesis Kit (Bio Rad,

Hercules, CA, USA) according to the manufacturers' instructions. Semi-quantitative PCR for GM-CSF (csf2, primers 5'-TCAAAGAAGCCCTGAACCTCC-3' and 5'-AATATCTTCAGGCGGGTCTGC-3') and housekeeping gene rpl19 (primers: 5'-AGGCATATGGGCATAGGGAAG-3' and 5'-TTGACCTTCAGGTACAGGCTGT-3') was performed in a 7300 PCR thermal cycler (Applied Biosystems, Carlsbad, CA, USA). PCR products were loaded on a 2% agarose gel (Lonza, Basel, Switzerland). *Tissue:* Snap-frozen aortas were homogenized in QIAzol (Qiagen, Venlo, Netherlands) and spleens were homogenized in RLT buffer with 1% β-mercaptoethanol followed by RNA extraction with the RNeasy Mini Kit (Qiagen, Venlo, Netherlands). Quantitative real-time TaqMan PCR for Tbet/Tbx21 (Mm00450960_m1; Applied Biosystems), Foxp3 (Mm00475162_m1; Applied Biosystems), GATA3 (Mm00484683_m1; Applied Biosystems), RORγt (Mm01261022_m1; Applied Biosystems), IFNγ (Mm01168134_m1; Applied Biosystems), IL-4 (Mm00445259_m1; Applied Biosystems), IL-10 (Mm00439614_m1; Applied Biosystems), IL-12b (Mm00434174_m1; Applied Biosystems), IL-17a (Mm00439618_m1; Applied Biosystems), TGFβ1 (Mm01178820_m1; Applied Biosystems), Csf2 (Mm01290062_m1; Applied Biosystems) and housekeeping gene β-actin (4352341E; Applied Biosystems) was run on a 7500 PCR thermal cycler (Applied Biosystems).

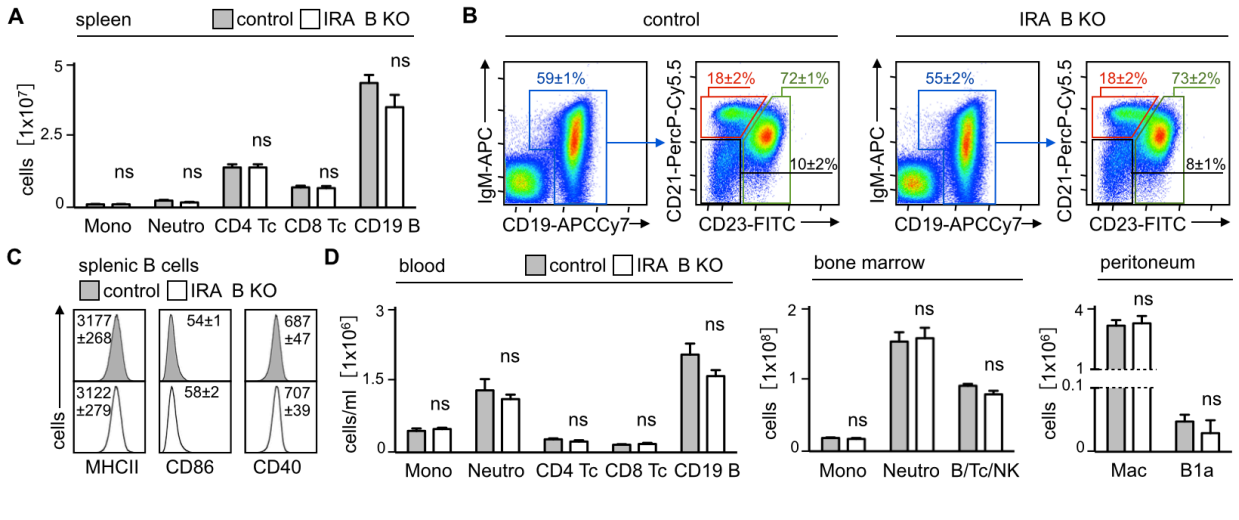
Cell culture. Bone marrow cells from CD45.1$^+$ mice were incubated with anti-CD3-Biotin (clone 145-2C11, Biolegend), anti-CD90.2-Biotin (clone 53-2.1, BD Biosciences), anti-CD19-Biotin (clone 6D5, Biolegend), anti-B220-Biotin (clone RA3-6B2, BD Biosciences), anti-NK1.1-Biotin (clone PK136, BD Biosciences), anti-Ly6G-Biotin (clone 1A8, Biolegend) Ab at 4 µl/1 x 10^8 cells followed by incubation with 100 µl anti-Biotin MACS beads (Miltenyi Biotec) per 1 x 10^8 cells. After passing through a Midi MACS separator and LS column negatively selected cells were counted in the lineage depleted flow-through. 7 x 10^5 Lin$^-$ bone marrow cells were cultured in 1 ml RPMI-1640 supplemented with 10% FBS, 25 mM HEPES, 2mM L- glutamine, 50 µM β-mercaptoethanol, 100 U/ml penicillin, 100 U/ml streptomycin (complete medium) in a 24 well plate (Cellgro). 7 x10^5 LPS induced IRA B cells and corresponding *Csf2*$^{-/-}$ B cells, respectively, were added to the culture together with 5000 U/ml murine IL-4 (Peprotech, Rocky Hill, NJ, USA) at day 0 and day 5 with replacing medium. Adherent cells were harvested after 8 days of culture, counted and stained for Dendritic cell (DC) marker expression with flow cytometric antibodies. In analogy 1 x10^5 IRA B cells (IgM^{high}, CD23^{low}, CD43^{high}, CD138^{high}) isolated from atherosclerotic *Ldlr*$^{-/-}$ mice (3 months on HCD) were co-culture with 1 x10^5 Lin$^-$ bone marrow cells in a flat-bottom 96-well plate. For T cell proliferation assays 1 x 10^4 WT IRA B cell generated bone marrow derived DC (BMDC) were transferred into U-shaped wells of a 96-well plate and loaded with 100 µg/ml ovalbumin (OVA; Sigma-Aldrich A7641) or 100 µg/ml BSA in a final volume of 200 µl/well. *Ldlr*$^{-/-}$ IRA B cell generated BMDC were kept in the flat-bottom 96-well plate and loaded with 100 µg/ml OVA or BSA. Sorted CD4$^+$ CD25$^-$ T cells from OT-II mice were stained with Cell Tracer Violet (Life Technologies) according to the manufacturer's instructions. Thereafter 5 x 10^4 OT-II CD4 T cells were added to each well and harvested after 4 days of co-culturing for flow cytometric assessment of proliferation cycles. For T_{reg} suppression assay C57Bl/6J splenocytes were incubated with anti-CD90.2-Biotin Ab (clone 53-2.1, BD Bioscience) at 4 µl/1 x 10^8 cells followed by incubation with 100 µl anti-Biotin MACS beads (Miltenyi Biotec) per 1 x 10^8 cells. After passing through a Midi MACS separator and LS column negatively selected cells were counted in the flow-through and resuspended in complete medium. T cell-depleted splenocytes were transferred to a U-bottom 96-well plate (3 x 10^5 cells/well) and irradiated with 30Gy prior to loading with 1 µg/ml anti-CD3e (clone 145-2C11, ebioscience) where indicated. Sorted CD45.1$^+$ CD4$^+$ CD25$^-$ T_{conv} cells were labeled with Cell Tracer Violet (Life Technologies) according to the manufacturer's instructions and 3 x 10^4 T_{conv} cells were added to 3 x 10^5 irradiated T cell depleted splenocytes in a total volume of 200µl/well. Sorted CD45.2$^+$ CD4$^+$ CD25$^+$ T_{reg} cells were added to T_{conv} cells at varying ratios (T_{conv} : T_{reg} = 1:0, 27:1, 9:1, 3:1, 1:1, 1:2). Cells were harvested after 3 days of co-culturing for flow cytometric assessment of proliferation cycles in CD45.1$^+$ T_{conv} cells.

Supplemental Figures and Figure Legends



	no CVD	CVD
number of patients	4	4
mean age ± SEM	62 ± 4	71 ± 6
gender	2 female, 2 male	1 female, 3 male
reason for spleen examination	suspicion for tumor in neighboring organs	autopsy after myocardial infarction; 1 tumor

Supplemental Figure 1: IRA B cell phenotype and ontogeny. (A) Surface marker expression profile of IRA B cells isolated from spleens of LPS challenged C57Bl/6 (4 x 25mg/day LPS i.p.) and atherosclerotic *Ldlr*$^{-/-}$ and *Apoe*$^{-/-}$ mice (3 months on HCD). GM-CSF^{+} leukocytes are depicted in red and GM-CSF^{-} leukocytes in grey. **(B)** Isotype staining controls on human spleen sections. **(C)** Characteristics of patients with and without cardiovascular disease (CVD). **(D)** *Ldlr*$^{-/-}$ mice were lethally irradiated, reconstituted with a 50:50 mixture of CD45.1^{+} WT and CD45.2^{+} *Myd88*$^{-/-}$ bone marrow and placed on HCD for 3 months. **(E)** Discrimination of *Myd88*$^{-/-}$ and *Myd88*$^{+/+}$ (WT) splenocytes based on CD45.2 and CD45.1 staining and flow cytometry. Further staining for GM-CSF, B220 and IgM allowed for identification of GM-CSF^{+} IRA B cells in the CD45.1^{+} WT but not the CD45.2^{+} *Myd88*$^{-/-}$ B cell population. Representative dot plots are shown for one of three mixed chimeras).

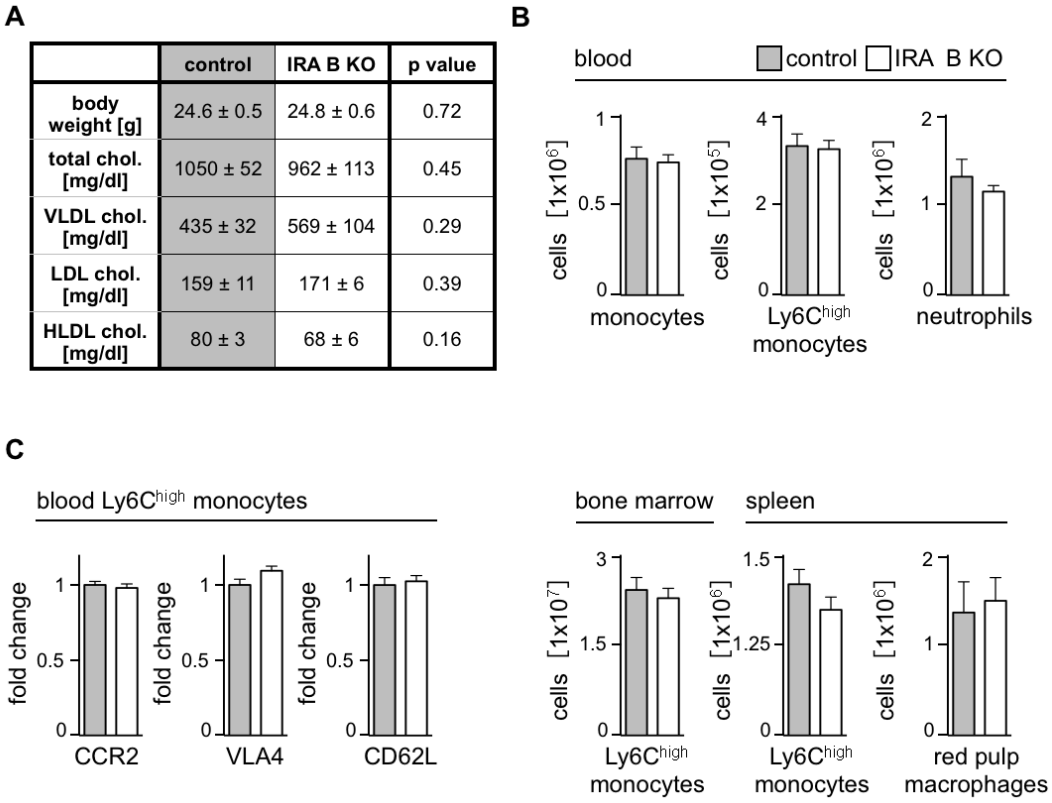


	Control		IRA B KO		
GM-CSF⁺ non-B cells	Mean %	SEM	Mean %	SEM	p value
CD4+ T cell	45.31	1.59	40.74	2.72	0.17
CD8+ T cell	20.94	1.84	18.84	2.29	0.49
neutrophil	6.55	1.32	9.61	2.7	0.33
Ly6C^{high} monocyte	3.44	0.83	4.86	0.84	0.25
Ly6C^{low} monocyte	1.67	0.22	1.44	0.13	0.38
cDC	0.96	0.14	0.9	0.11	0.76
other	21.22	4.29	23.64	3.99	0.69

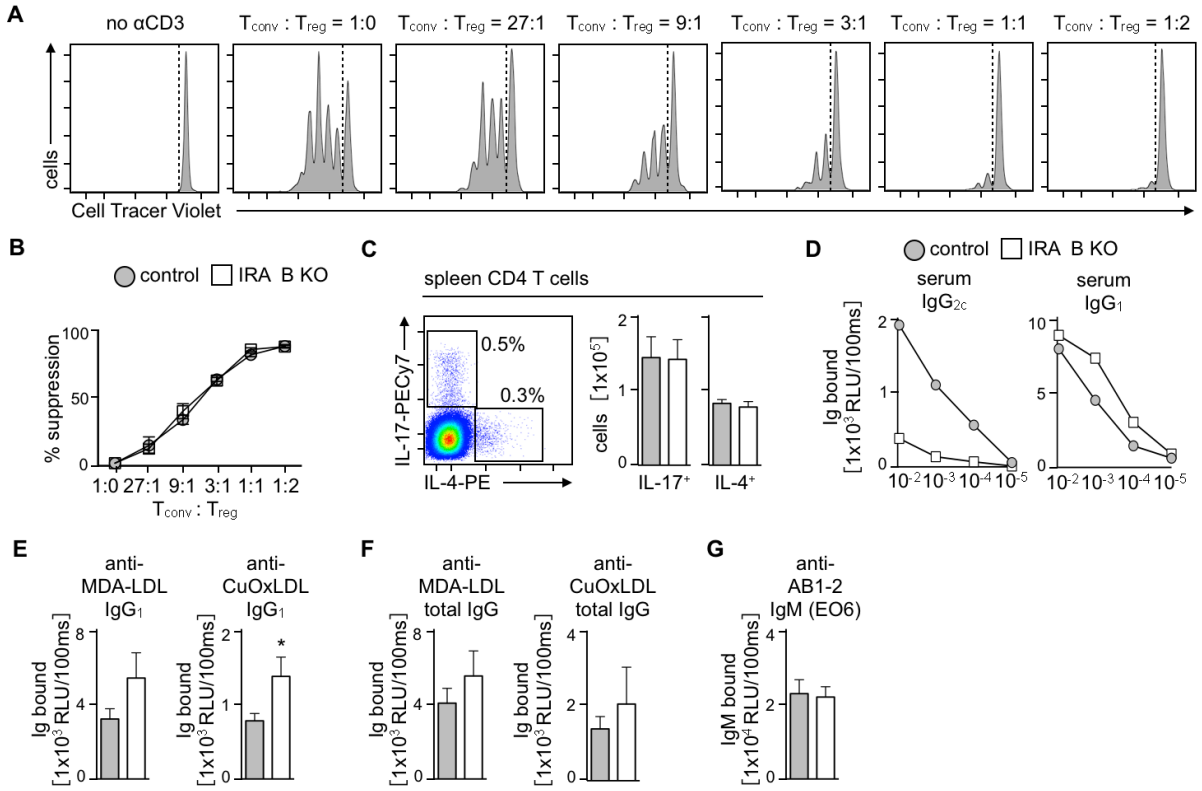
Supplemental Figure 2: Leukocyte subsets in reconstituted mixed bone marrow chimeras. (A) Enumeration of monocytes (Mono), neutrophils (Neutro), CD4$^+$ and CD8$^+$ T cells (CD4 Tc, CD8 Tc), CD19$^+$ B cells (CD19 B) in the spleen of control (gray) and IRA B KO (white) mice 6 weeks after reconstitution. Results are presented as mean ± SEM, n = 6 per group., **(B)** Identification and quantification of splenic B cell subsets in both groups 6 weeks after reconstitution. The CD21^{high} CD23^{low} population contains marginal zone B cells, CD21^{low} CD23^{low} cells encompass B1 and plasma cells, and B2/T2 cells are CD23^{high}. Results are presented as mean ± SEM, n = 6 per group, controls in gray and IRA B KO mice in white. **(C)** Splenic B cell expression of antigen presenting and costimulatory molecules MHCII, CD86 and CD40 as determined by flow cytometry. Results are presented as mean fluorescence intensity (MFI) ± SEM, n = 6 per group. **(D)** Leukocyte subsets in blood, spleen, bone marrow and peritoneum of control (gray) and IRA B KO (white) mice 6 weeks after reconstitution. Results are presented as mean ± SEM, n = 6 per group. **(E)** Quantification of GM-CSF (*Csf2)* expression in whole spleen tissue of IRA B KO (white) and control (gray) mice by real-time PCR. Results are presented as mean ± SEM fold change of $2^{\Delta Ct}$, ** $p \leq 0.01$, $n \geq 20$ per group. **(F)** Relative contribution of non-B cells to leukocyte derived GM-CSF production as assessed by flow cytometry. Results are presented as mean ± SEM, n = 7 per group.

A

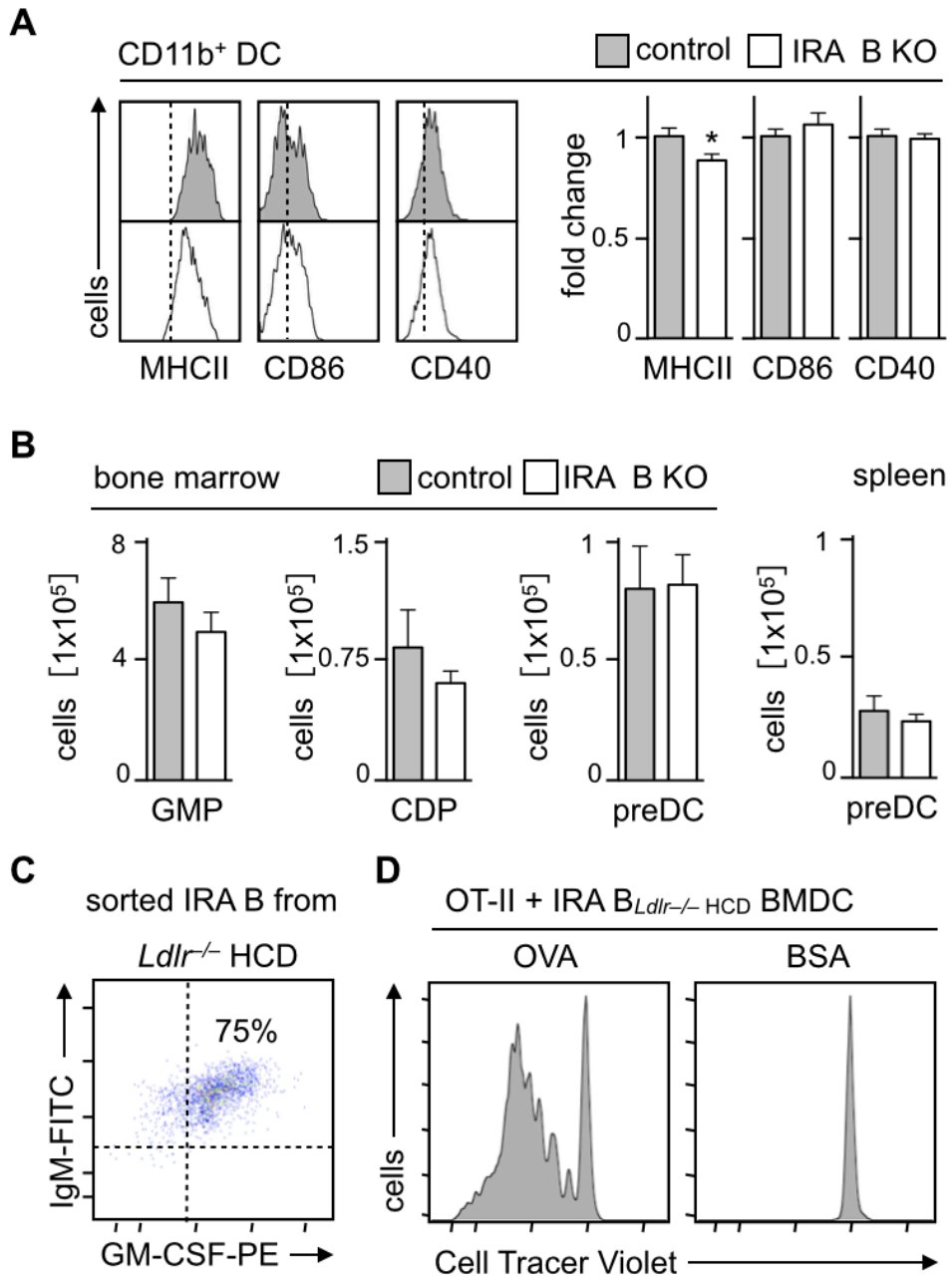
	control	IRA B KO	p value
body weight [g]	24.6 ± 0.5	24.8 ± 0.6	0.72
total chol. [mg/dl]	1050 ± 52	962 ± 113	0.45
VLDL chol. [mg/dl]	435 ± 32	569 ± 104	0.29
LDL chol. [mg/dl]	159 ± 11	171 ± 6	0.39
HLDL chol. [mg/dl]	80 ± 3	68 ± 6	0.16



Supplemental Figure 3: IRA B cell deficiency does not affect monocytosis and hypercholesterolemia. (A) Measurement of total body weight, serum levels of total cholesterol ($n \geq 10$ per group) and VLDL, LDL and HDL cholesterol (3 pooled samples of $n \geq 6$ mice per group) after 10 week HCD feeding. Results are presented as mean ± SEM. **(B)** Enumeration of total monocytes, Ly6C^{high} monocytes, neutrophils and CD11b^{low} F4/80^{high} red pulp macrophages in blood, spleen or bone marrow after 10 week HCD feeding. Results are presented as mean ± SEM, $n \geq 10$ per group, * $p \leq 0.05$, controls in gray and IRA B KO mice in white. **(C)** Expression of CCR2, VLA4 and CD62L on Ly6C^{high} blood monocytes after 10 week HCD feeding as determined by flow cytometry. Quantification of MFI presented as mean ± SEM fold change between control (gray) and IRA B KO (white) mice, $n \geq 10$ per group.



Supplemental Figure 4: IRA B cells influence T_{H1} cell-dependent anti-OxLDL isotype switching but not T_{reg} suppressive function. (A) Regulatory T cell (T_{reg}) suppression assay. WT $CD4^+$ $CD25^-$ conventional T cell (T_{conv}) were co-cultured with $CD4^+$ $CD25^+$ T_{reg} cells sorted from IRA B KO and control mice after 10 week HCD feeding at increasing dilutions as indicated. Representative histograms show proliferation of cell tracer dye-labeled T_{conv} cells in response to soluble anti-CD3e (1 μg/ml) and T cell-depleted, irradiated spleen stimulator cells under the suppressive influence of T_{reg} cells. **(B)** Dose-dependent quantification of T_{reg}-induced suppression of T_{conv} proliferation. Results are presented as mean ± SEM, n = 4 per group, controls in gray circles and IRA B KO mice in white squares. **(C)** On the left representative dot plot showing intracellular staining of $CD3^+$ $CD4^+$ T cells for IL-4 and IL-17. On the right quantification of IL-4-producing T_{H2} and IL-17-producing T_{H17} cells in spleens of IRA B KO (white) and control (gray) mice after 10 week HCD feeding. Results are presented as mean ± SEM, n = 7 per group. **(D)** Antibody binding dilution curves for total serum IgG_{2c} and IgG_1 antibodies. Results are presented as mean for triplicates of pooled samples, $n \geq 6$ per group, controls in gray circles and IRA B KO mice in white squares. (**E, F**) Quantification of IgG_1 and total IgG antibody titers against MDA-LDL and copper-oxidized LDL (CuOxLDL) in 1:25 diluted individual serum samples, $n \geq 10$ per group. Results are presented as mean ± SEM, * $p \leq 0.05$, controls in gray and IRA B KO mice in white. **(G)** Quantification of anti-AB1-2-IgM (EO6) antibody titers in 1:25 diluted individual serum samples, $n \geq 10$ per group. Results are presented as mean ± SEM, controls in gray and IRA B KO mice in white.



Supplemental Figure 5: IRA B cell deficiency does not affect generation of cDC progenitors. (**A**) Expression of MHCII, CD86, and CD40 on $CD8^{-}$ $CD11b^{+}$ splenic cDC as determined by flow cytometry in IRA B KO (white) and control (gray) mice after 10 week HCD feeding. On the left representative histograms show mean fluorescence intensities (MFI) for MHCII, CD86 and CD40 compared to isotype controls (dashed line). On the right quantification of MFI presented as mean ± SEM fold change between control (gray) and IRA B KO (white) mice, $n \geq 10$ per group, * $p \leq 0.05$. (**B**) Enumeration of granulocyte-macrophage progenitors (GMP), common dendritic cell progenitor (CDP) and preDC precursor in bone marrow and spleen after 10 week HCD feeding. Results are presented as mean ± SEM, $n \geq 10$ per group, controls in gray and IRA B KO mice in white. (**C**) Flow assisted cell sorting of $CD23^{low}$ IgM^{high} $CD43^{high}$ $CD138^{high}$ ($IRAB_{Ldlr-/-\ HCD}$) cells from atherosclerotic *Ldlr*$^{-/-}$ mice (3 months on HCD). Dashed lines represent isotype controls. (**D**) $CD4^{+}$ $CD25^{-}$ OT-II cells were co-cultured

with IRA $B_{Ldlr-/-\ HCD}$ cell-generated BMDC loaded with chicken ovalbumin (100μg/ml) or BSA (100μg/ml) for 4 days. Representative histograms show cell divisions of $CD4^+$ OT-II cells labeled with a cell tracer dye.

Supplemental References:

1. Colvin GA, Lambert JF, Abedi M, Hsieh CC, Carlson JE, Stewart FM, Quesenberry PJ. Murine marrow cellularity and the concept of stem cell competition: geographic and quantitative determinants in stem cell biology. *Leukemia*. 2004;18:575-583.
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