Original Research

Artery Tertiary Lymphoid Organs Control Multilayered Territorialized Atherosclerosis B-Cell Responses in Aged *ApoE*−/− Mice

Prasad Srikakulapu,* Desheng Hu,* Changjun Yin,* Sarajo K. Mohanta,* Sai Vineela Bontha,
Li Peng, Michael Beer, Christian Weber, Coleen A. McNamara, Gianluca Grassia,
Pasquale Maffia, Rudolf A. Manz,* Andreas J.R. Habenicht*

Objective—Explore aorta B-cell immunity in aged *ApoE*−/− mice.

Approach and Results—Transcript maps, fluorescence-activated cell sorting, immunofluorescence analyses, cell transfers, and Ig-ELISPOT assays showed multilayered atherosclerosis B-cell responses in artery tertiary lymphoid organs (ATLOs). Aging-associated aorta B-cell–related transcriptomes were identified, and transcript atlases revealed highly territorialized B-cell responses in ATLOs versus atherosclerotic lesions: ATLOs showed upregulation of bona fide B-cell genes, including Cd19, Ms4a1 (Cd20), Cd79a/b, and Ighm although intima plaques preferentially expressed molecules involved in non–B effector responses toward B-cell–derived mediators, that is, Fcgr3 (Cd16), Fcer1g (Cd23), and the C1q family. ATLOs promoted B-cell recruitment. ATLO B-2 B cells included naive, transitional, follicular, germinal center, switched IgG1+, IgA+, and IgE+ memory cells, plasmablasts, and long-lived plasma cells. ATLOs recruited large numbers of B-1 cells whose subtypes were skewed toward interleukin-10+ B-1b cells versus interleukin-10− B-1a cells. ATLO B-1 cells and plasma cells constitutively produced IgM and IgG and a fraction of plasma cells expressed interleukin-10. Moreover, *ApoE*−/− mice showed increased germinal center B cells in renal lymph nodes, IgM-producing plasma cells in the bone marrow, and higher IgM and anti–MDA-LDL IgG serum titers.

Conclusions—ATLOs orchestrate dichotomic, territorialized, and multilayered B-cell responses in the diseased aorta; germinal center reactions indicate generation of autoimmune B cells within the diseased arterial wall during aging. (***Arterioscler Thromb Vasc Biol***. 2016;36:00-00. DOI: 10.1161/ATVBAHA.115.306983.)

Key Words: aging ■ atherosclerosis ■ B-lymphocytes ■ germinal center ■ inflammation

Beyond their ability to produce antibodies,[1] B cells produce proinflammatory or anti-inflammatory cytokines,[2,3] present antigen to T cells,[4] and regulate B- and T-cell responses.[5] Mature naive bone marrow (BM)–derived B-2 cells home into SLOs where they undergo somatic hypermutation and affinity maturation in germinal centers (GCs). Antigen-experienced B-2 cells either become short-lived plasma cells (PCs) residing in SLOs or they develop into long-lived PCs that largely home to the BM.[6–8] By contrast, the majority of B-1 cells are located in the peritoneal cavity (PerC) and pleural cavities where they form a pool of quiescent innate B cells. On migration to inflammatory tissues, B-1 cells become activated and self-renew to carry out T-cell–independent protective immune responses.[9–12] Recent reports showed differential effects of B-cell subsets in atherosclerosis[13–24] with antiatherogenic effects of B-1 cells and proatherogenic effects of B-2

Nonstandard Abbreviations and Acronyms	
ATLOs	artery tertiary lymphoid organs
BM	bone marrow
FACS	fluorescence-activated cell sorting
GC	germinal center
PCs	plasma cells
PerC	peritoneal cavity
RLNs	renal lymph nodes
WT	wild-type

cells.[25–27] In addition to SLOs and the BM, B-cell responses may be organized in artery tertiary lymphoid organs (ATLOs) in *ApoE*^{−/−} mice.[28,29] Here, we report on local aorta as opposed to systemic B-cell responses during aging.

Materials and Methods

Materials and Methods are available in the online-only Data Supplement.

Results

Aorta B-Cell Transcripts During Aging

MIAME-compliant microarrays were prepared as described[30,31]; data were deposited in the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) and the gene ontology (http://www.geneontology.org/) data banks (accession GSE40156).[30,32] To determine if B-cell–related gene expression changes with aging, microarrays of aortas, SLOs, and blood from wild-type (WT) and *ApoE*^{−/−} mice were compared. B-cell–related genes were altered in WT aortas during aging (Table I in the online-only Data Supplement). However, there were much more pronounced changes in *ApoE*^{−/−} when compared with WT aortas. Expression kinetics of some of these genes correlated with the kinetics of ATLO formation[32,33] (Figure 1; Table I in the online-only Data Supplement). B-cell transcriptomes contained genes that were expressed exclusively by B cells and a majority of genes that respond to B-cell–derived molecules yielding a complex B-cell immunity–related gene map (Figure 1; Table I in the online-only Data Supplement). Examples of the magnitude of B-cell immunity–related transcripts in *ApoE*^{−/−} aortas include a 135-fold increase of Ighm (IgM constant region), a 29-fold increase in Ptpn6 (protein tyrosine phosphatase, nonreceptor type 6; SHP1) regulating the IgM repertoire, a 23-fold increase in the immunosuppressive Lilrb3 (leukocyte immunoglobulin-like receptor, subfamily B with TM and ITIM domains), Fcer1g (Fc receptor, IgE, high-affinity I, γ-polypeptide), and Cd28 (CD28 antigen) expression that promotes PC survival (Figure 1; Table I in the online-only Data Supplement). In contrast, spleen- and blood-transcript maps were considerably smaller, and the extent of differential expression between WT and *ApoE*^{−/−} mice was much less pronounced (Figure I in the online-only Data Supplement). The majority of B-cell–associated genes in the spleen and blood were downregulated during aging in both WT and *ApoE*^{−/−} mice: Ptprc (B220; Cd45; protein tyrosine phosphatase, receptor type, C) involved in cell fate

decisions of the B-cell receptor; Aicda (activation-induced cytidine deaminase) regulating somatic hypermutation and Ig class switching; Sykb (spleen tyrosine kinase) participating in B-memory cell survival; Vav3 (Vav3 oncogene) mediating B-cell receptor responses; Tcf3 (transcription factor 3) controlling B-cell ontogeny; Foxp1 (forkhead box p1) impacting B-cell survival; and Malt1 (Malt1 paracaspase) participating in B-cell malignancies. In summary, the spleen and blood gene maps suggested that age-associated changes largely mirrored B-cell senescence rather than genotype/hyperlipidemia-dependent changes (Figure I and Table I in the online-only Data Supplement).

Transcript Maps Delineate the Territoriality of B-Cell–Related Immune Responses in the Aged *ApoE*^{−/−} Aorta

Laser capture microdissection aorta-derived tissues were obtained together with renal lymph nodes (RLNs) and spleen.[30,31] B-cell–related genes were expressed at higher levels in ATLOs when compared with aorta adventitia segments from WT or *ApoE*^{−/−} mice without plaques (Figure 2A; Table I in the online-only Data Supplement). In the adventitia cluster, genes associated with B-cell survival, proliferation, differentiation, and activation, such as immunoglobulin genes (ighm), TACI (tnfrsf13b), BAFF receptor (tnfrsf13c), CD40 antigen (cd40), histocompatibility 2, class II antigen A, β-1 (h2-ab1), complement components (c1qb), and Myd88 (myd88) were robustly expressed in adventitial regions adjacent to plaques compared with adventitia in regions with no plaques (Figure 2A; Table I in the online-only Data Supplement). Moreover, the adventitia adjacent to plaques contained transcripts coding for Igj chain (immunoglobulin joining chain; Igj) involved in somatic hypermutation and memory B-cell development; CD79a (immunoglobulin-associated α; Ly54) involved in B-cell receptor signaling; and Ms4a1 (CD20) controlling T-cell–dependent humoral immunity (Figure IIA in the online-only Data Supplement). The plaque–ATLO cluster markedly expressed Cd19 (CD19 antigen) in ATLOs involved in B-cell maturation, Cd20, Igj chain, Igm, and Cd79a/b (Figure 2B; Figure IIB in the online-only Data Supplement). In addition, the plaque–ATLO B-cell cluster[30,31] showed functional separation in B-cell–related genes in ATLOs versus plaques: bona fide B-cell genes displayed strong expression in ATLOs versus low expression in plaques. For example, Ighm, cd19, ms4a1 (cd20), Igj, and cd79a/b were expressed manifold higher in ATLOs when compared with plaques, which expressed genes that respond to B-cell products (Figure 2A; Figure IIB and Table I in the online-only Data Supplement). In contrast, the transcript atlas showed almost identical levels of B-cell–related genes in WT versus *ApoE*^{−/−} spleens, RLNs, and blood (Figure I in the online-only Data Supplement; Figure 2C and 2D). It is also noticeable that the LN–ATLO cluster shows a comparably higher expression in ATLOs versus LNs of innate immune response genes, such as fcgr1, fcgr2b, fcgr3, c4b, and the c1q family, indicating ongoing inflammation in ATLOs (Figure 2C and 2D).
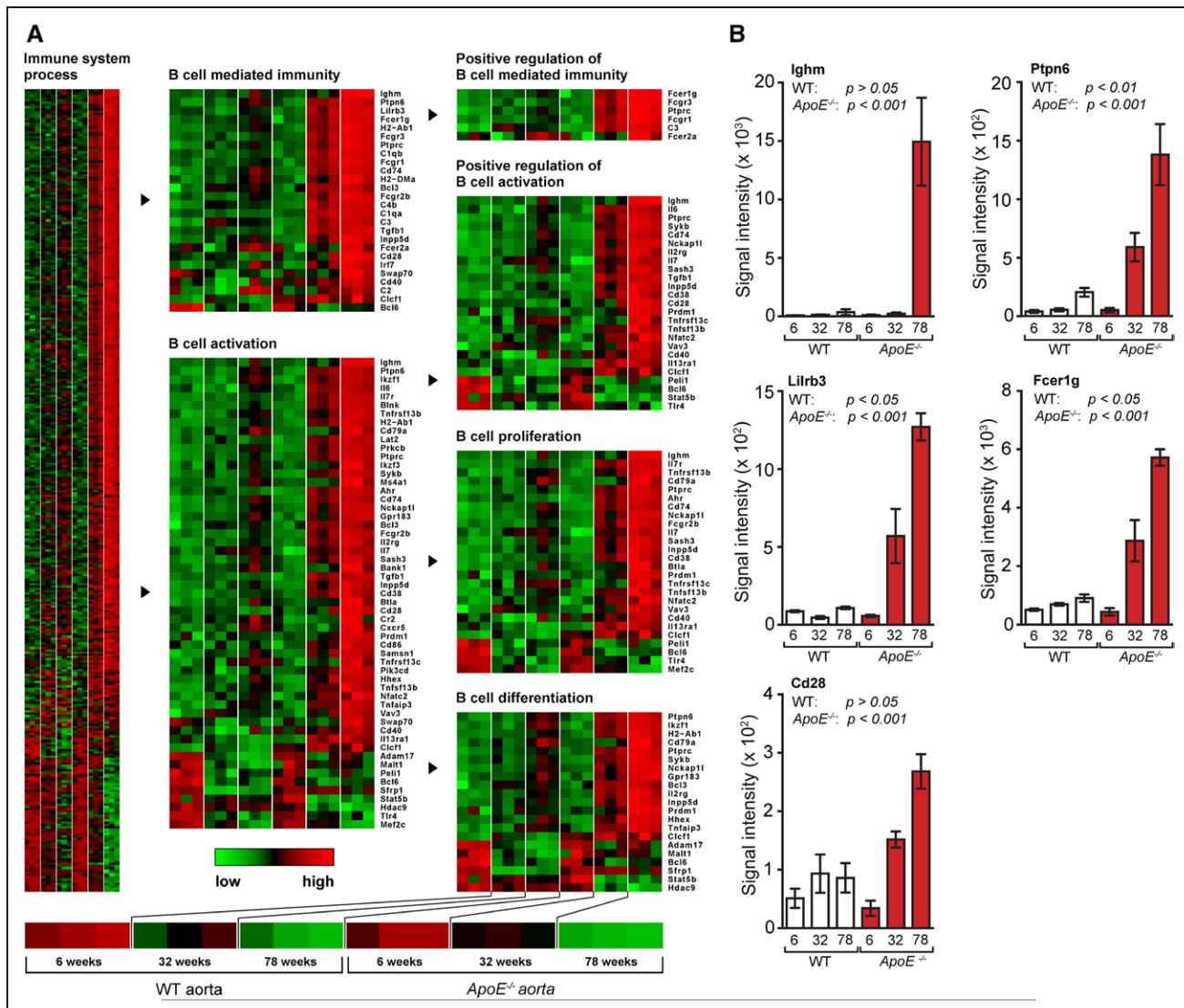
Figure 1. Aging-associated changes in aorta B-cell immunity. **A,** Age-associated transcript profiles of wild-type (WT) and *ApoE*⁻/⁻ aorta of 6-, 32-, and 78-week-old mice (3 mice per genotype per age group). Transcripts in gene ontology terms immune system process, B-cell–mediated immunity, B-cell activation, positive regulation of B-cell–mediated immunity, positive regulation of B-cell activation, B-cell proliferation, and B-cell differentiation are displayed as heatmaps. **B,** Expression of selected genes in aorta from WT and *ApoE*⁻/⁻ mice at 6, 32, and 78 weeks; n=3 mice per genotype per age group. Results represent mean±SEM. Analyses were performed using ANOVA with Benjamini–Hochberg correction. Absolute numbers of signal intensities and statistics are reported in Table I in the online-only Data Supplement.

ATLO B-2 Subtypes Suggest Antigen-Specific GC Reactions

B cells present in the aorta of aged *ApoE*⁻/⁻ mice predominantly reside in ATLOs, whereas they cannot be observed in plaques of young WT or *ApoE*⁻/⁻ mice.[30,32,33] Fluorescence-activated cell sorting (FACS) analyses of B cells revealed the magnitude of differences in ATLOs and WT adventitia; and B220 immunostaining confirmed that B cells are located in ATLOs and in the adjacent draining LNs but none in WT adventitia or plaques (Figure 3A and 3B). Considerable numbers of T-/B-cell clusters referred to as fat-associated lymphoid clusters were observed in paraaortic adipose tissue of aged *ApoE*⁻/⁻ mice and numerous small paraaortic LNs containing B cells lined the tissue adjacent to the adventitia (not shown). There were no differences in the frequency of B cells

in SLOs or blood of WT versus *ApoE*⁻/⁻ mice (Figure 3C). To obtain evidence for an ongoing GC reaction in ATLOs, CD19, IgM, and IgD antisera together with FACS gating for 4 different populations from CD19⁺ B cells were used (Figure 3D). IgM⁺/IgD⁻, IgM⁺/IgD⁺, IgM⁻/IgD⁻, and IgM⁻/IgD⁺ B cells were identified in abdominal but not thoracic aorta segments: IgM⁺/IgD⁻ cells represent either immature or transitional B cells (also referred to as T-1 cells) representing the earliest B-cell stage present outside the BM or these cells may represent B-1 B cells[34]; IgM⁺/IgD⁺ and IgM⁻/IgD⁺ cells represent mature B-cell stages.[35,36] Among mature IgD⁺ cells, IgM⁻/IgD⁺ are mature follicular B-2 cells.[37] IgM⁻/IgD⁻ cells represent either switched Ig⁺ B cells, GC B cells that have transiently lost Ig expression when undergoing hypermutation of their Ig genes or GC-derived memory B cells.[34,38]
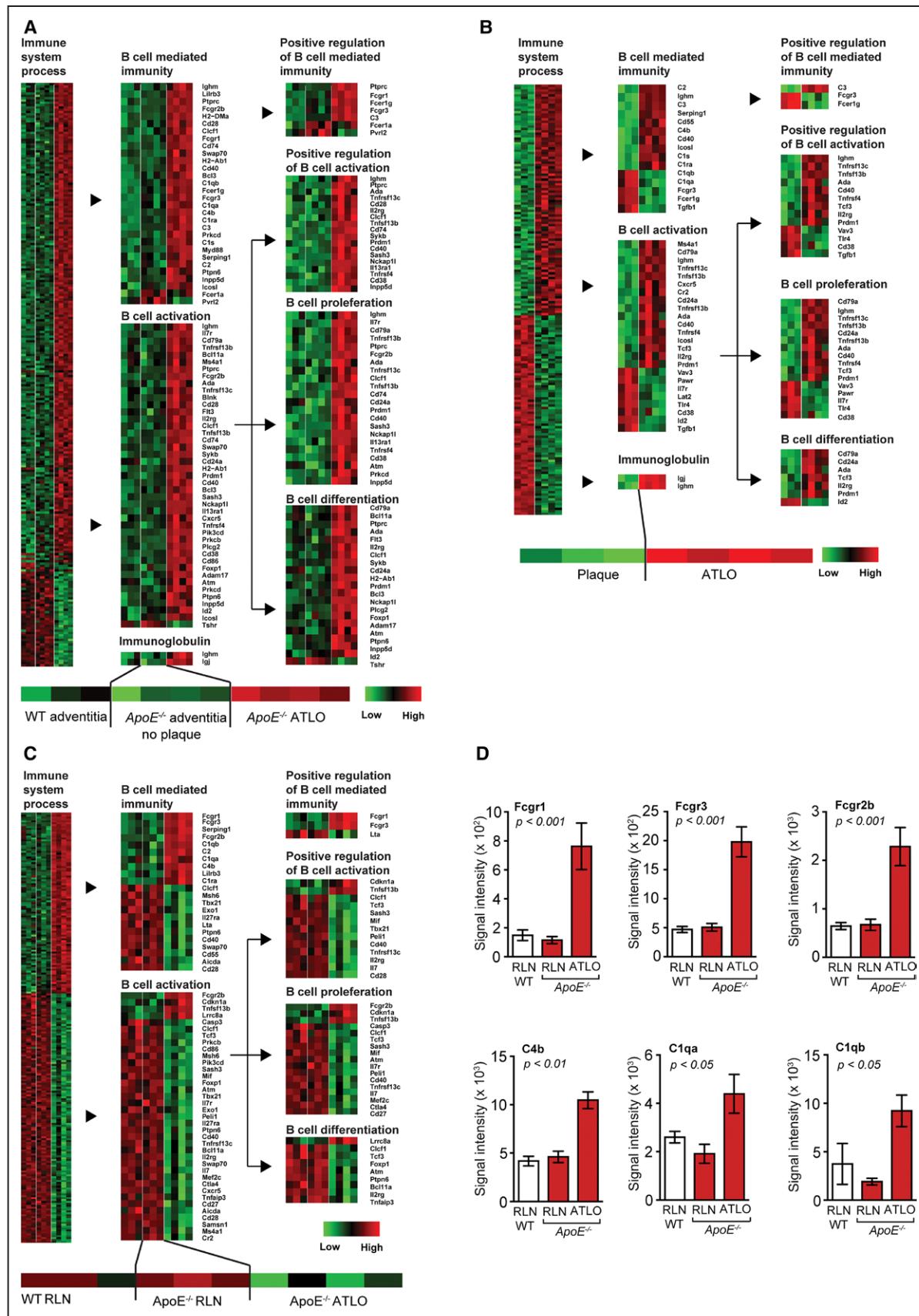
Figure 2. Aorta transcript maps reveal the specificity and territoriality of B-cell–related immune responses in artery tertiary lymphoid organs (ATLOs). **A**, Heatmaps of differentially expressed genes in the adventitia cluster (wild-type [WT], n=3; $ApoE^{-/-}$, n=4). **B**, Plaque/ATLO cluster (for plaque, n=3; for ATLOs, n=4). **C**, Lymph node (LN) cluster (for WT and $ApoE^{-/-}$ LNs, n=3; for ATLO, n=4); (Continued)

None of the subsets were found in the abdominal aorta of WT mice (Figure 3D). WT and *ApoE*⁻/⁻ SLOs and blood revealed equivalent numbers of these subsets with the exception of an increase in transitional IgM⁺/IgD⁻ B cells in RLNs of *ApoE*⁻/⁻ versus WT mice (Figure 3E). We determined the percentages of IgM⁺/IgD⁺ or switched Ig⁺ B cells in SLOs, blood, WT aortas, and ATLOs. SLO and blood IgM⁺/IgD⁺ and switched Ig⁺ B cells were similar in WT and *ApoE*⁻/⁻ SLOs (Figure 3F and 3G). Although undetectable in WT adventitia, the percentage of IgM⁺/IgD⁺ B cells in ATLOs
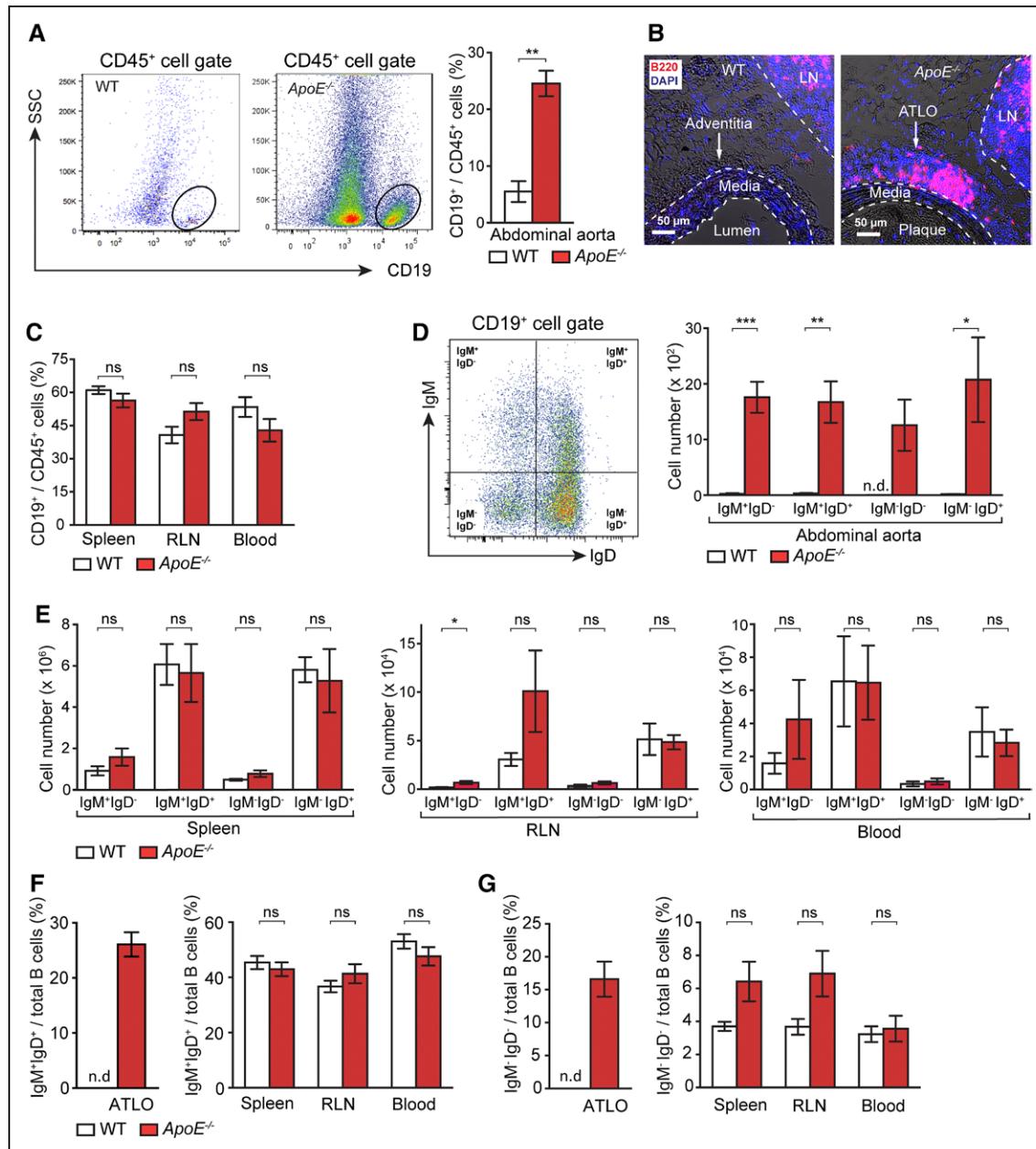
Figure 3. Artery tertiary lymphoid organs (ATLOs) harbor a diverse repertoire of B-cell subsets. **A,** Flow cytometry of CD19⁺ B cells of total CD45⁺ leukocytes in abdominal aorta of 80-week-old wild-type (WT) and *ApoE*⁻/⁻ mice (WT, n=11; *ApoE*⁻/⁻, n=10). **B,** Immunofluorescence staining with anti-B220 antisera shows B cells in ATLOs and lymph nodes (LNs) but none in the media (M) or plaque (P) or in WT adventitia. **C,** B cells in the spleen, renal LNs (RLNs), and blood of WT and *ApoE*⁻/⁻ mice (WT, n=8; *ApoE*⁻/⁻, n=6). Flow cytometric analysis of IgM⁺IgD⁻, IgM⁺IgD⁺, IgM⁻IgD⁻, and IgM⁻IgD⁺ B cells per total CD19⁺ B cells in abdominal aorta (**D**) and spleen, RLN, and blood of 80-week-old WT and *ApoE*⁻/⁻ mice (WT n=4; *ApoE*⁻/⁻ n=5; **E**). Percentages of IgM⁺IgD⁺ B cells (**F**) and IgM⁻IgD⁻ B cells per total B cells in ATLOs, spleen, RLN, and blood of age-matched WT and *ApoE*⁻/⁻ mice (**G**). Results represent mean±SEM; *P<0.05, **P<0.01, and ***P<0.001; 2-sided unpaired Student *t* test. n indicates the number of experiments; n.d., not detectable; and ns, not significant.

approached that in SLOs (Figure 3F). However, the percentage of switched Ig$^+$ B cells in ATLOs exceeded those in SLOs or blood (Figure 3G). We determined the number of B-1 cells in the PerC and of plasmablasts and PCs in the abdominal aorta, spleen, and RLNs of *ApoE$^{-/-}$* mice (Figure III in the online-only Data Supplement). No change in B-1 B cell subtypes was observed in the PerC of WT versus *ApoE$^{-/-}$* mice (Figure IIIA in the online-only Data Supplement). Moreover, aged *ApoE$^{-/-}$* abdominal aortas, spleens, and RLNs contained

plasmablasts and PCs; some of which expressed interleukin (IL)-10 (Figure IIIB in the online-only Data Supplement).

ATLOs Harbor GC B Cells and IgG1$^+$, IgA$^+$, and IgE$^+$ Memory Cells

Naive B cells in SLOs enter GCs to undergo a GC reaction involving somatic hypermutation and affinity maturation of their BCRs. ATLO GC B cells were identified by FACS (IgD$^-$/PNA$^+$/GL-7$^+$): they were undetectable in WT aortas but ranged at ≈9%
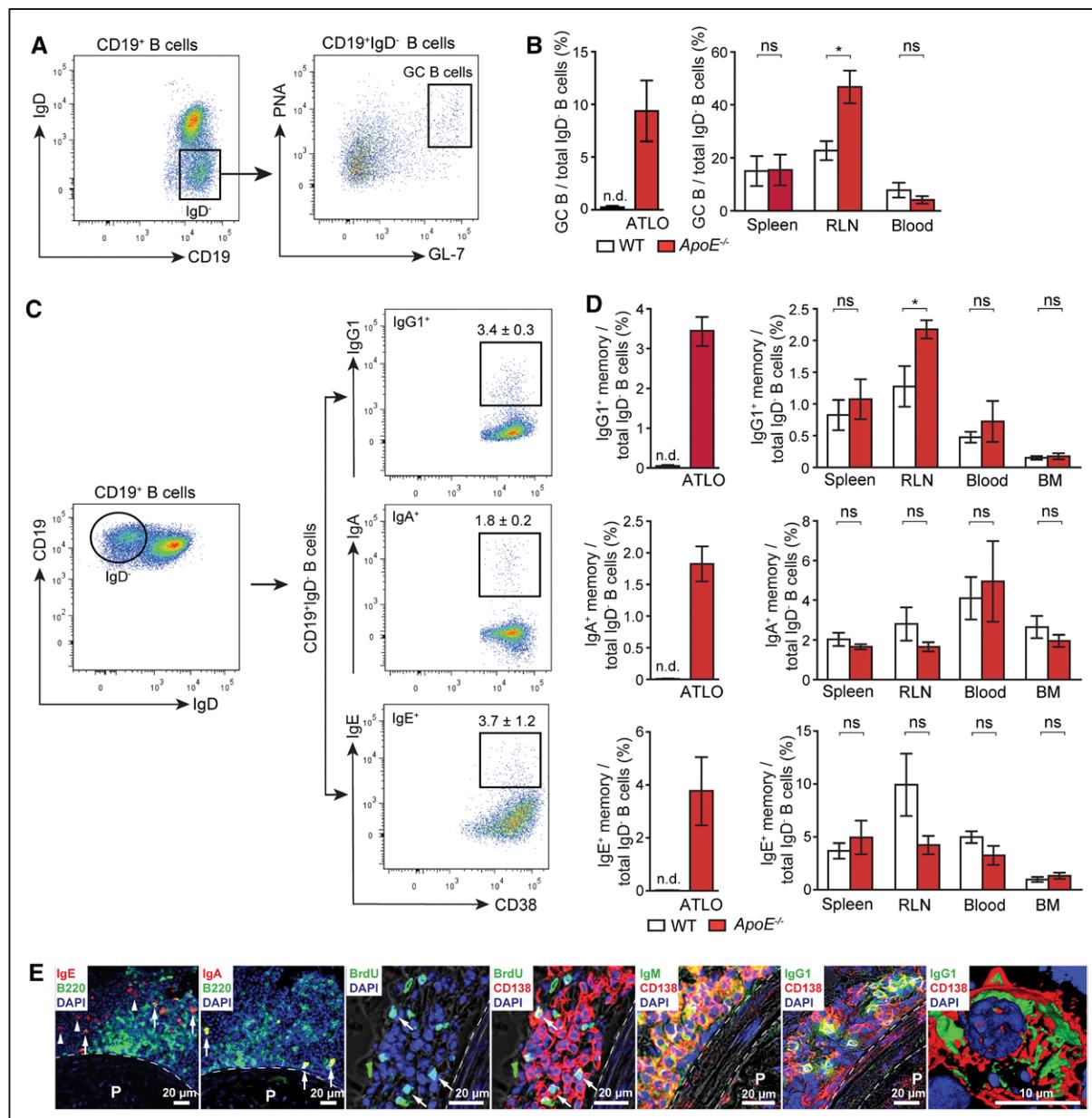
Figure 4. Artery tertiary lymphoid organs (ATLOs) contain B cells that participate in humoral immune responses. **A**, IgD$^-$ B cells gated from CD19$^+$ total B cells were evaluated for PNA$^+$/GL-7$^+$ (germinal center [GC] B cells) in ATLOs. **B**, GC B cells in ATLOs and SLOs were quantified (wild-type [WT], n=4; *ApoE$^{-/-}$*, n=5). **C**, IgG1$^+$ (IgG1$^+$/CD38$^+$), IgA$^+$ (IgA$^+$/CD38$^+$), and IgE$^+$ (IgE$^+$/CD38$^+$) memory B cells were gated from total CD19$^+$/IgD$^-$ B cells. **D**, Quantification of IgG1$^+$, IgA$^+$, and IgE$^+$ memory B cells in ATLOs, SLOs, blood, and bone marrow (BM; WT, n=4; *ApoE$^{-/-}$*, n=4). Results represent mean±SEM; *$P<0.05$, 2-sided unpaired Student t test. **E**, Immunofluorescence data of IgE$^+$ memory B cells (IgE$^+$/B220$^+$ indicated with arrows and IgE$^+$/B220$^-$ cells indicated with arrow heads), IgA$^+$ memory B cells (IgA$^+$/B220$^+$), long-lived plasma cells (PCs; CD138$^+$/BrdU$^-$) and short-lived PCs (CD138$^+$/BrdU$^+$; white arrow), IgM- (IgM$^+$/CD138$^+$), and IgG1- (IgG1$^+$/CD138$^+$) producing PCs in ATLOs. Dotted line outlines media. n indicates the number of experiments; n.d., not detectable; ns, not significant; P, plaque, and RLN, renal lymph node.

of all IgD^{-} B cells in ATLOs (Figure 4A and 4B). Their number was similar in WT and *ApoE*^{-/-} spleen and blood although they were more abundant in *ApoE*^{-/-} when compared with WT RLNs (Figure 4B). We sought evidence for isotype-switching using FACS analyses. Surprisingly, we observed significant numbers of CD19^{+}/IgD^{-}/IgG1^{+}, CD19^{+}/IgD^{-}/IgA^{+}, and CD19^{+}/IgD^{-}/IgE^{+} B cells in ATLOs (Figure 4C and 4D). Although class switching is not restricted to GCs, the presence of GCs and cells that class switched to T-dependent Ig subclasses, such as IgG1, suggests that these cells resemble memory B cells. Intriguingly, the percentage of IgD^{-} B cells that class switched to IgG1 was significantly greater than those in the spleen, RLNs, BM, or blood (Figure 4D). In contrast, there were equivalent percentages of IgG1^{+} B cells in the spleen, BM, and blood of WT versus *ApoE*^{-/-} mice. Consistent with rare ATLO formation in the thoracic aorta,[32] no GC B cells or class switched B cells were observed there (not shown). These data provide evidence for a disease-specific antigen-dependent B-2 B cell maturation pathway in ATLOs.

Short-Lived and Long-Lived PCs in ATLOs

Long-lived PCs are major constituents of humoral memory. Long-lived PCs preferentially home to the BM, whereas short-lived PCs remain within SLOs. Nothing is known about PCs in atherosclerosis. As long-lived PCs survive for long periods of time in the BM,[39] we determined the composition of ATLO PC subtypes.[8] Both long-lived and short-lived PCs were observed in ATLOs (Figure 4E).[40,41] Moreover, survival factors for long-lived PCs, including CXCL12, BAFF,[39] and others, are markedly expressed in ATLOs[30,32] (Table I in the online-only Data Supplement).

ATLOs Promote B-2 and B-1 Cell Recruitment Into the Arterial Wall

To determine B-cell recruitment by ATLOs, we adoptively transferred Ly5.1 B-2 cells to aged Ly5.2 WT or *ApoE*^{-/-} mice. After 36 hours, B-2 cells had migrated predominantly to the abdominal aorta of *ApoE*^{-/-} mice (Figure 5A and 5B) although none were recruited to WT aortas. Comparably low but similar numbers of

Figure 5. Artery tertiary lymphoid organs (ATLOs) promote B-2 B-cell recruitment into the abdominal aorta, skewing of ATLO B-1 cells toward B-1b cells. Fluorescence-activated cell sorting (FACS)–purified Ly5.1^{+} CD19^{+}/CD43^{-} B-2 cells (purity, >98%) were adoptively transferred via tail vein injection into aged wild-type (WT) or *ApoE*^{-/-} mice. Thirty-six hours later, Ly5.2[+] recipient mice were analyzed for B-2 cell migration into ATLOs or thoracic aorta segments. **A**, Migrated B-2 B cells were gated from total B cells in ATLOs. **B**, Quantification of migrated Ly5.1[+] B-2 B cells in aorta. **C**, Peritoneal cavity (PerC), spleen, renal lymph nodes (RLNs), and bone marrow (BM). Results represent mean±SEM; *P<0.05, 2-sided unpaired Student t test. WT, n=3; *ApoE*^{-/-}, n=3. B-1 cells selectively accumulate in ATLOs. **D**, IgM^{hi}/CD43^{+} B-1 cells were gated from CD19^{+} B cells, and CD5^{+} B-1a and CD5^{-} B-1b cells were gated from total B-1 cells in ATLOs and the percentage of B-1 cells from total B cells were quantified in ATLOs and SLOs. **E**, Absolute numbers of B-1 cells were quantified in aortic segments in WT and *ApoE*^{-/-} mice. The ratio of B-1b/B-1a B cells in ATLOs compared with that in PerC of WT and *ApoE*^{-/-} mice. FACS plots show the gating strategy for B-cell subpopulations in PerC (**F**) and their frequencies of B cells in CD45^{+} cells, B-1, and B-2 cells in total B cells, B-1a, and B-1b cells in B-1 cells were compared between WT and *ApoE*^{-/-} mice (**G**). Results represent mean±SEM; **P<0.01 and ***P<0.001; 2-sided unpaired Student t test with Bonferroni–Holm correction. WT and *ApoE*^{-/-}, n=5–6. n indicates the number of experiments; n.d., not detectable; and ns, not significant.

B-2 cells were recruited into the PerCs of WT and *ApoE*^{−/−} mice. There was no difference in B-cell recruitment into the spleen, RLNs, and BM of WT versus *ApoE*^{−/−} mice (Figure 5C). Similar data were obtained with B-1 cells (not shown).

B-1 Cells Accumulate in ATLOs and Are Skewed Toward B-1b Cells

B-1 cells are predominantly located in body cavities.[42,43] Recent studies showed that B-1a cells reside in the aorta perivascular tissue of young *ApoE*^{−/−} mice.[22] To determine if B-1 cells are located in the aged aorta adventitia, we performed FACS analyses. A high percentage of all B cells, that is, ≈21%, in ATLOs were B-1 cells (Figure 5D), and their relative contribution to all B cells exceeded that in the spleen and RLNs by a large margin (Figure 5D). The reason for B-1 B-cell accumulation is most likely the high expression of CXCL13 in ATLOS. Numbers of total B-1 cells in ATLOs

are comparable with that of IgM[+]/IgD^{−} cells, indicating that most IgM[+]/IgD^{−} cells found in this compartment are B-1 cells. The abdominal aorta harbored considerably higher numbers of B-1 cells when compared with the thoracic aorta (Figure 5E). The B-1 subtype composition was aberrant as we observed a high number of B-1b versus B-1a cells, which dramatically differs from that relation in the PerC (Figure 5E).[9] There was no significant difference in total B cells, B-2, B-1a, and B-1b cells in the PerC of aged WT and *ApoE*^{−/−} mice (Figure 5F and 5G).

Majority of ATLO B-1b but Not B-2 Cells Express IL-10, PD-L1, FasL, and Transforming Growth Factor-β

In view of skewing of ATLO B-1 cells toward the B-1b subtype (Figure 5D and 5E) and a recent report showing that B-1b cells protect against atherosclerosis,[21] we searched for
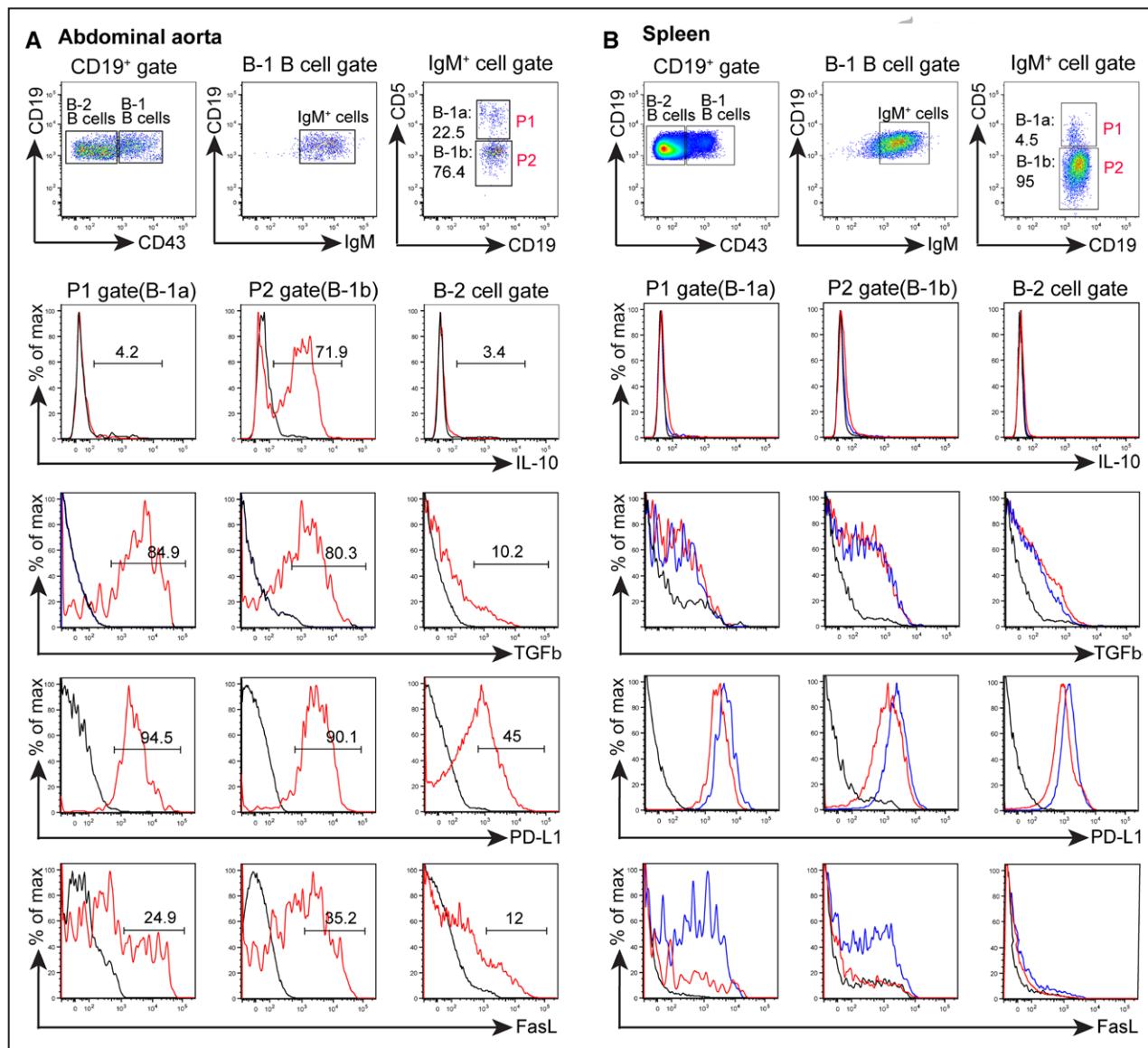
Figure 6. Artery tertiary lymphoid organ (ATLO) B-1 B cells show a predominant immunosuppressive IL-10^{+}/PD-L1[+]/FasL[+]/TGFβ[+] phenotype. Cell suspensions from individual aged *ApoE*^{−/−} mice. **A**, IL-10^{+}, TGFβ[+], PD-L1[+], and FasL[+] abdominal aorta B cells. **B**, *ApoE*^{−/−} (red) spleen (80- to 85-week old mice) and WT (blue); *ApoE*^{−/−} (n=3–4). B-1a, B-1b, and B-2 cell populations were gated and assayed for cytokine expression (or isotype control, black). Numbers designate frequencies of positive cells.

mechanisms of immunosuppression within the arterial wall. IL-10–producing B-1a rather than B-1b or B-2 cells were found in the PerC (Figure IIIA in the online-only Data Supplement). However, we observed that the majority (≈72%) of abdominal aorta B-1b cells produced IL-10 though a minor component of B-1a cells and a significant but low proportion of IL-10+ cells in the thoracic aorta (not shown). No or comparably low numbers of B-1a cells or B-2 cells expressed IL-10 (Figure 6A). Moreover, the frequency of IL-10+ B cells in ATLOs is higher than those of their counterparts in the spleen and RLNs of WT or *ApoE*−/− mice (Figure 6B). Following a report that a subset of PCs secretes IL-10,[44] we assessed IL-10 expression in PCs. A significant proportion of ATLO CD138+/CD19+ plasmablasts were IL-10+ PCs (Figure IIIB in the online-only Data Supplement). Similar PCs have been shown to suppress immune responses in disease models.[45] We further assessed

the phenotype of B-1 cells in the abdominal aorta. ATLO B-1 but to a much lesser extent B-2 cells expressed PD-L1, FasL, and transforming growth factor-β, indicating that these cells exert immunosuppressive functions (Figure 6A).

Ig-Secreting Cells Accumulate in ATLOs

ELISPOT experiments were performed. There were no constitutively IgM- and IgG-secreting cells in either the thoracic or abdominal aorta of WT mice (Figure 7A and 7B). Few IgM- and IgG-secreting cells were observed in the thoracic aorta of *ApoE*−/− mice (Figure 7A and 7B). However, ATLOs contained abundant IgM- and IgG-secreting cells amounting to ≤80-fold increase of IgM-secreting B cells and a 24-fold increase in IgG-secreting B cells in the abdominal aorta (Figure 7A and 7B). Blood contains few (<10 cells per 0.5 mL of blood) IgM- or IgG-secreting cells (data not shown). In the
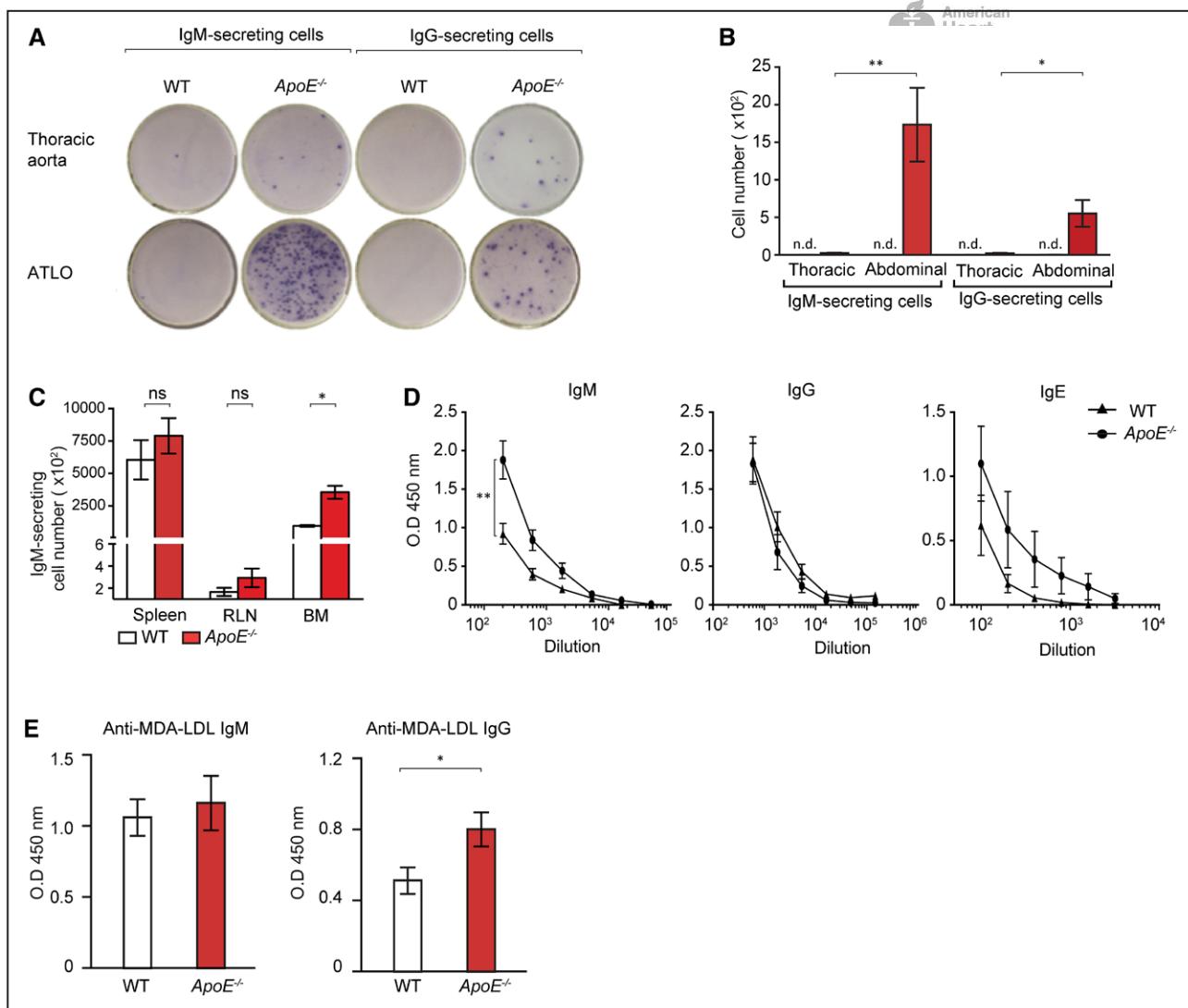
Figure 7. IgM- and IgG-secreting B cells are selectively located in artery tertiary lymphoid organs (ATLOs). **A**, ELISPOT analyses of IgM- and IgG-secreting B cells in ATLOs and thoracic aorta segments. **B**, Quantification of IgM- and IgG-secreting cells in ATLOs versus thoracic aorta. **C**, Quantification of IgM-secreting cells in the spleen, renal lymph node (RLN) and bone marrow (BM) of age-matched wild-type (WT) and *ApoE*−/− mice. **D**, Serum titers of IgM, IgG, and IgE in aged WT and *ApoE*−/− mice. **E**, Anti–MDA-LDL IgM and anti–MDA-LDL IgG serum titers (dilution factor 10 and 25, respectively) in aged WT and *ApoE*−/− mice. Results represent mean±SEM; 2-sided unpaired Student *t* test; n=10 per genotype; *P<0.05 and **P<0.01. ns indicates not significant.

spleen and RLNs, there was no difference in Ig-secreting cells between WT and *ApoE*$^{-/-}$ mice (Figure 7C). However, IgM-secreting cells were higher in *ApoE*$^{-/-}$ BM when compared with WT BM raising the possibility of a systemic PC response in *ApoE*$^{-/-}$ mice. To examine a systemic B-cell response, we determined serum titers of IgM, IgG, and IgE, as well as anti–MDA-LDL IgM and IgG. Aged *ApoE*$^{-/-}$ mice had significantly higher levels of total IgM but not IgG or IgE levels when compared with aged WT mice (Figure 7D). Although anti–MDA-LDL IgM levels were not different, anti–MDA-LDL IgG levels were significantly higher in *ApoE*$^{-/-}$ versus WT mice (Figure 7E).

Discussion

These data identify ATLOs as the principal lymphoid tissue that orchestrates atherosclerosis B-cell immunity during aging of *ApoE*$^{-/-}$ mice. Atherosclerosis ATLO B-cell responses are specific, robust, highly territorialized, multilayered, and include a comprehensive adaptive B-2 and a substantial aberrant innate B-1 cell component: ATLOs but not WT adventitia harbor an unusual set of class-switched IgG1$^+$, IgA$^+$, and IgE$^+$ B cells, a significant number of IL-10$^+$/PD-L1$^+$/FasL$^+$/TGFβ$^+$ B-1b cells, and both short-lived and long-lived PCs, including a fraction of IL-10$^+$ PCs. This body of data—together with our previous observation that B cells are major constituents of ATLO antigen-presenting cells[30]—reveal a yet unrecognized scenario of aorta atherosclerosis-specific B-cell immunity, which includes B effector cells, PCs, and several immunosuppressive B-cell subtypes (Figure IV in the online-only Data Supplement).

ATLO B-2 B-cell subtypes include transitional, follicular, GC, and IgG1$^+$, IgA$^+$, and IgE$^+$ B cells—the latter representing class-switched B cells and PCs. These data are the first to suggest that (auto)antigen-dependent hypermutation, proliferation, affinity maturation, Ig class switching, memory cell generation, and differentiation into long-lived PCs may be carried out in the arterial wall. It is becoming evident that ATLOs provide a new paradigm of atherosclerosis-specific B-cell immunity and possibly autoimmunity: ATLO B-cell responses occur in aged animals, whereas aortas of young *ApoE*$^{-/-}$ or young and aged WT mice do not show a significant aorta B-cell compartment.[30,46–48] It should be pointed out, however, that this study falls short of proving antigen-specific ATLO-dependent autoimmune B-2 B-cell generation. In this regard, the observation of a considerable number of PCs in ATLOs deserves special attention: PCs may arise from B-1 cells, from B-2 cells via T-cell–independent mechanisms, or from B-2 cells via T-cell–dependent mechanisms.[49] Further studies on the origin of aorta PCs seem warranted as the role of PCs in atherosclerosis remains unknown.

Our data demonstrate that local B-cell immune subsets can be distinguished from those in SLOs, the PerC, and the BM: their aberrant nature manifests itself by the presence of large numbers of IL-10$^+$ B-1b cells, of short-lived and long-lived PCs, and of IL-10$^+$ PCs. Possibly, our aged mice will allow to isolate B cells from ATLOs and SLOs to compare their BCR repertoire. Moreover, the accumulation of IgA$^+$ and IgE$^+$ B cells in the diseased aorta indicates links of atherosclerosis B-cell immunity to innate inflammatory leukocytes in plaques. IgA, IgE, and IgG act through either activating or inhibitory

Fc receptors on virtually all innate immune cells, including macrophages.[50] The expression of divergent Fc receptors raises the possibility that Fc receptors may be involved in the dichotomic control of inflammation within diseased arteries: Fcer1g (Cd23) is a high-affinity IgE receptor that is upregulated during aging, and Fcgr1 (Cd64), Fcgr2b (Cd32), and Fcgr3 (Cd16) are prominently expressed in ATLOs.

ATLOs contain multiple B-cell subtypes, including IgM$^+$/IgD$^-$, IgM$^+$/IgD$^+$, IgM$^-$/IgD$^-$, and IgM$^-$/IgD$^+$ B cells. ATLO IgM$^+$/IgD$^-$ and IgM$^+$/CD43$^+$ B cells may be B-1 cells. In addition, the presence of class-switched memory B cells suggests that some ATLO IgM$^+$/IgD$^-$ B cells may represent IgM$^+$ memory B cells that have not undergone class switching. IgM$^+$ memory B cells considerably contribute to the total population of all memory B cells.[51] Whether the population of IgM$^+$/IgD$^-$ B cells within ATLOs also includes a fraction of immature or transitional B cells that represent the earliest B-cell stages that are found outside the BM is a possibility that deserves attention. Under physiological conditions, immature B cells immigrate from the BM and specifically home to splenic follicles to undergo differentiation into a transitional B-cell stage and finally either become mature B-2 or marginal zone B cells.[52] This final B-cell maturation is accompanied by a shift of the BCR repertoire that includes counterselection against autoreactive cells that occurs in discrete and tightly controlled steps.[53] Hence, it is tempting to speculate that immature B cells home to ATLOs to undergo differentiation into mature B cells in the absence of the proper control mechanisms acting in the spleen: this could allow for the generation of autoreactive atherosclerosis-specific B cells.

Disclosures

None.

References

1. Kurosaki T, Kometani K, Ise W. Memory B cells. *Nat Rev Immunol*. 2015;15:149–159. doi: 10.1038/nri3802.
2. Browning JL. B cells move to centre stage: novel opportunities for autoimmune disease treatment. *Nat Rev Drug Discov*. 2006;5:564–576. doi: 10.1038/nrd2085.
3. Shen P, Fillatreau S. Antibody-independent functions of B cells: a focus on cytokines. *Nat Rev Immunol*. 2015;15:441–451. doi: 10.1038/nri3857.
4. Yuseff MI, Pierobon P, Reversat A, Lennon-Duménil AM. How B cells capture, process and present antigens: a crucial role for cell polarity. *Nat Rev Immunol*. 2013;13:475–486. doi: 10.1038/nri3469.
5. Mauri C, Bosma A. Immune regulatory function of B cells. *Annu Rev Immunol*. 2012;30:221–241. doi: 10.1146/annurev-immunol-020711-074934.

6. Allman D, Pillai S. Peripheral B cell subsets. *Curr Opin Immunol*. 2008;20:149–157. doi: 10.1016/j.coi.2008.03.014.

7. Hardy RR, Kincade PW, Dorshkind K. The protean nature of cells in the B lymphocyte lineage. *Immunity*. 2007;26:703–714. doi: 10.1016/j.immuni.2007.05.013.

8. Hoyer BF, Moser K, Hauser AE, Peddinghaus A, Voigt C, Eilat D, Radbruch A, Hiepe F, Manz RA. Short-lived plasmablasts and long-lived plasma cells contribute to chronic humoral autoimmunity in NZB/W mice. *J Exp Med*. 2004;199:1577–1584. doi: 10.1084/jem.20040168.

9. Baumgarth N. The double life of a B-1 cell: self-reactivity selects for protective effector functions. *Nat Rev Immunol*. 2011;11:34–46. doi: 10.1038/nri2901.

10. Haas KM, Poe JC, Steeber DA, Tedder TF. B-1a and B-1b cells exhibit distinct developmental requirements and have unique functional roles in innate and adaptive immunity to S. pneumoniae. *Immunity*. 2005;23:7–18. doi: 10.1016/j.immuni.2005.04.011.

11. Alugupalli KR, Leong JM, Woodland RT, Muramatsu M, Honjo T, Gerstein RM. B1b lymphocytes confer T cell-independent long-lasting immunity. *Immunity*. 2004;21:379–390. doi: 10.1016/j.immuni.2004.06.019.

12. Roy B, Shukla S, Łyszkiewicz M, Krey M, Viegas N, Düber S, Weiss S. Somatic hypermutation in peritoneal B1b cells. *Mol Immunol*. 2009;46:1613–1619. doi: 10.1016/j.molimm.2009.02.026.

13. Caligiuri G, Nicoletti A, Poirier B, Hansson GK. Protective immunity against atherosclerosis carried by B cells of hypercholesterolemic mice. *J Clin Invest*. 2002;109:745–753. doi: 10.1172/JCI7272.

14. Major AS, Fazio S, Linton MF. B-lymphocyte deficiency increases atherosclerosis in LDL receptor-null mice. *Arterioscler Thromb Vasc Biol*. 2002;22:1892–1898.

15. Ait-Oufella H, Herbin O, Bouaziz JD, Binder CJ, Uyttenhove C, Laurans L, Taleb S, Van Vré E, Esposito B, Vilar J, Sirvent J, Van Snick J, Tedgui A, Tedder TF, Mallat Z. B cell depletion reduces the development of atherosclerosis in mice. *J Exp Med*. 2010;207:1579–1587. doi: 10.1084/jem.20100155.

16. Kyaw T, Tay C, Khan A, Dumouchel V, Cao A, To K, Kehry M, Dunn R, Agrotis A, Tipping P, Bobik A, Toh BH. Conventional B2 B cell depletion ameliorates whereas its adoptive transfer aggravates atherosclerosis. *J Immunol*. 2010;185:4410–4419. doi: 10.4049/jimmunol.1000033.

17. Kyaw T, Tay C, Hosseini H, Kanellakis P, Gadowski T, MacKay F, Tipping P, Bobik A, Toh BH. Depletion of B2 but not B1a B cells in BAFF receptor-deficient ApoE mice attenuates atherosclerosis by potently ameliorating arterial inflammation. *PLoS One*. 2012;7:e29371. doi: 10.1371/journal.pone.0029371.

18. Sage AP, Tsiantoulas D, Baker L, Harrison J, Masters L, Murphy D, Loinard C, Binder CJ, Mallat Z. BAFF receptor deficiency reduces the development of atherosclerosis in mice–brief report. *Arterioscler Thromb Vasc Biol*. 2012;32:1573–1576. doi: 10.1161/ATVBAHA.111.244731.

19. Galkina E, Kadl A, Sanders J, Varughese D, Sarembock IJ, Ley K. Lymphocyte recruitment into the aortic wall before and during development of atherosclerosis is partially L-selectin dependent. *J Exp Med*. 2006;203:1273–1282. doi: 10.1084/jem.20052205.

20. Doran AC, Lipinski MJ, Oldham SN, et al. B-cell aortic homing and atheroprotection depend on Id3. *Circ Res*. 2012;110:e1–12. doi: 10.1161/CIRCRESAHA.111.256438.

21. Rosenfeld SM, Perry HM, Gonen A, Prohaska TA, Srikakulapu P, Grewal S, Das D, McSkimming C, Taylor AM, Tsimikas S, Bender TP, Witztum JL, McNamara CA. B-1b cells secrete atheroprotective IgM and attenuate atherosclerosis. *Circ Res*. 2015;117:e28–e39. doi: 10.1161/CIRCRESAHA.117.306044.

22. Perry HM, Oldham SN, Fahl SP, Que X, Gonen A, Harmon DB, Tsimikas S, Witztum JL, Bender TP, McNamara CA. Helix-loop-helix factor inhibitor of differentiation 3 regulates interleukin-5 expression and B-1a B cell proliferation. *Arterioscler Thromb Vasc Biol*. 2013;33:2771–2779. doi: 10.1161/ATVBAHA.113.302571.

23. Clement M, Guedj K, Andreata F, et al. Control of the T follicular helper-germinal center B-cell axis by CD8+ regulatory T cells limits atherosclerosis and tertiary lymphoid organ development. *Circulation*. 2015;131:560–570. doi: 10.1161/CIRCULATIONAHA.114.010988.

24. Gjurich BN, Taghavie-Moghadam PL, Ley K, Galkina EV. L-selectin deficiency decreases aortic B1a and Breg subsets and promotes atherosclerosis. *Thromb Haemost*. 2014;112:803–811. doi: 10.1160/TH13-10-0865.

25. Lichtman AH, Binder CJ, Tsimikas S, Witztum JL. Adaptive immunity in atherogenesis: new insights and therapeutic approaches. *J Clin Invest*. 2013;123:27–36. doi: 10.1172/JCI63108.

26. Tsiantoulas D, Diehl CJ, Witztum JL, Binder CJ. B cells and humoral immunity in atherosclerosis. *Circ Res*. 2014;114:1743–1756. doi: 10.1161/CIRCRESAHA.113.301145.

27. Harmon DB, Srikakulapu P, Kaplan JL, et al. Protective role for B-1b B cells and IgM in obesity-associated inflammation, glucose intolerance, and insulin resistance. *Arterioscler Thromb Vasc Biol*. 2016;36:682–691. doi: 10.1161/ATVBAHA.116.307166.

28. Mohanta SK, Yin C, Peng L, Srikakulapu P, Bontha V, Hu D, Weih F, Weber C, Gerdes N, Habenicht AJ. Artery tertiary lymphoid organs contribute to innate and adaptive immune responses in advanced mouse atherosclerosis. *Circ Res*. 2014;114:1772–1787. doi: 10.1161/CIRCRESAHA.114.301137.

29. Pitzalis C, Jones GW, Bombardieri M, Jones SA. Ectopic lymphoid-like structures in infection, cancer and autoimmunity. *Nat Rev Immunol*. 2014;14:447–462. doi: 10.1038/nri3700.

30. Hu D, Mohanta SK, Yin C, et al. Artery tertiary lymphoid organs control aorta immunity and protect against atherosclerosis via vascular smooth muscle cell lymphotoxin β receptors. *Immunity*. 2015;42:1100–1115. doi: 10.1016/j.immuni.2015.05.015.

31. Yin C, Mohanta S, Ma Z, Weber C, Hu D, Weih F, Habenicht A. Generation of aorta transcript atlases of wild-type and apolipoprotein E-null mice by laser capture microdissection-based mRNA expression microarrays. *Methods Mol Biol*. 2015;1339:297–308. doi: 10.1007/978-1-4939-2929-0_20.

32. Gräbner R, Lötzer K, Döpping S, et al. Lymphotoxin beta receptor signaling promotes tertiary lymphoid organogenesis in the aorta adventitia of aged ApoE-/- mice. *J Exp Med*. 2009;206:233–248. doi: 10.1084/jem.20080752.

33. Moos MP, John N, Gräbner R, Nossmann S, Günther B, Vollandt R, Funk CD, Kaiser B, Habenicht AJ. The lamina adventitia is the major site of immune cell accumulation in standard chow-fed apolipoprotein E-deficient mice. *Arterioscler Thromb Vasc Biol*. 2005;25:2386–2391. doi: 10.1161/01.ATV.0000187470.31662.fe.

34. Nduati EW, Ng DH, Ndungu FM, Gardner P, Urban BC, Langhorne J. Distinct kinetics of memory B-cell and plasma-cell responses in peripheral blood following a blood-stage *Plasmodium chabaudi* infection in mice. *PLoS One*. 2010;5:e15007. doi: 10.1371/journal.pone.0015007.

35. Inman CF, Murray TZ, Bailey M, Cose S. Most B cells in non-lymphoid tissues are naïve. *Immunol Cell Biol*. 2012;90:235–242. doi: 10.1038/icb.2011.35.

36. Hardy RR, Hayakawa K, Parks DR, Herzenberg LA, Herzenberg LA. Murine B cell differentiation lineages. *J Exp Med*. 1984;159:1169–1188.

37. Sadri N, Lu JY, Badura ML, Schneider RJ. AUF1 is involved in splenic follicular B cell maintenance. *BMC Immunol*. 2010;11:1. doi: 10.1186/1471-2172-11-1.

38. Hayakawa K, Ishii R, Yamasaki K, Kishimoto T, Hardy RR. Isolation of high-affinity memory B cells: phycoerythrin as a probe for antigen-binding cells. *Proc Natl Acad Sci U S A*. 1987;84:1379–1383.

39. Radbruch A, Muehlinghaus G, Luger EO, Inamine A, Smith KG, Dörner T, Hiepe F. Competence and competition: the challenge of becoming a long-lived plasma cell. *Nat Rev Immunol*. 2006;6:741–750. doi: 10.1038/nri1886.

40. Starke C, Frey S, Wellmann U, Urbonaviciute V, Herrmann M, Amann K, Schett G, Winkler T, Voll RE. High frequency of autoantibody-secreting cells and long-lived plasma cells within inflamed kidneys of NZB/W F1 lupus mice. *Eur J Immunol*. 2011;41:2107–2112. doi: 10.1002/eji.201041315.

41. Mumtaz IM, Hoyer BF, Panne D, Moser K, Winter O, Cheng QY, Yoshida T, Burmester GR, Radbruch A, Manz RA, Hiepe F. Bone marrow of NZB/W mice is the major site for plasma cells resistant to dexamethasone and cyclophosphamide: implications for the treatment of autoimmunity. *J Autoimmun*. 2012;39:180–188. doi: 10.1016/j.jaut.2012.05.010.

42. Hayakawa K, Hardy RR, Herzenberg LA, Herzenberg LA. Progenitors for Ly-1 B cells are distinct from progenitors for other B cells. *J Exp Med*. 1985;161:1554–1568.

43. Lalor PA, Stall AM, Adams S, Herzenberg LA. Permanent alteration of the murine Ly-1 B repertoire due to selective depletion of Ly-1 B cells in neonatal animals. *Eur J Immunol*. 1989;19:501–506. doi: 10.1002/eji.1830190314.

44. Shen P, Roch T, Lampropoulou V, et al. IL-35-producing B cells are critical regulators of immunity during autoimmune and infectious diseases. *Nature*. 2014;507:366–370. doi: 10.1038/nature12979.

45. Matsumoto M, Baba A, Yokota T, Nishikawa H, Ohkawa Y, Kayama H, Kallies A, Nutt SL, Sakaguchi S, Takeda K, Kurosaki T, Baba Y. Interleukin-10-producing plasmablasts exert regulatory function in

autoimmune inflammation. *Immunity*. 2014;41:1040–1051. doi: 10.1016/j.immuni.2014.10.016.

46. Kulkarni U, Karsten C, Kohler T, et al. IL-10 mediates plasmacytosis-associated immunodeficiency by inhibiting complement-mediated neutrophil migration. *J Allergy Clin Immunol*. 2015. doi: 10.1016/j.jaci.2015.10.018. In press.

47. Strom AC, Cross AJ, Cole JE, Blair PA, Leib C, Goddard ME, Rosser EC, Park I, Hultgårdh Nilsson A, Nilsson J, Mauri C, Monaco C. B regulatory cells are increased in hypercholesterolaemic mice and protect from lesion development via IL-10. *Thromb Haemost*. 2015;114:835–847. doi: 10.1160/TH14-12-1084.

48. Sage AP, Nus M, Baker LL, Finigan AJ, Masters LM, Mallat Z. Regulatory B cell-specific interleukin-10 is dispensable for atherosclerosis development in mice. *Arterioscler Thromb Vasc Biol*. 2015;35:1770–1773. doi: 10.1161/ATVBAHA.115.305568.

49. Hiepe F, Radbruch A. Plasma cells as an innovative target in autoimmune disease with renal manifestations. *Nat Rev Nephrol*. 2016;12:232–240. doi: 10.1038/nrneph.2016.20.

50. Nimmerjahn F, Ravetch JV. Antibody-mediated modulation of immune responses. *Immunol Rev*. 2010;236:265–275. doi: 10.1111/j.1600-065X.2010.00910.x.

51. Dogan I, Bertocci B, Vilmont V, Delbos F, Mégret J, Storck S, Reynaud CA, Weill JC. Multiple layers of B cell memory with different effector functions. *Nat Immunol*. 2009;10:1292–1299. doi: 10.1038/ni.1814.

52. Cancro MP. Peripheral B-cell maturation: the intersection of selection and homeostasis. *Immunol Rev*. 2004;197:89–101.

53. Loder F, Mutschler B, Ray RJ, Paige CJ, Sideras P, Torres R, Lamers MC, Carsetti R. B cell development in the spleen takes place in discrete steps and is determined by the quality of B cell receptor-derived signals. *J Exp Med*. 1999;190:75–89.

Highlights

- Artery tertiary lymphoid organs (ATLOs) orchestrate B-cell responses in the diseased aorta.
- ATLOs promote B-1 and B-2 cell recruitment into the arterial wall.
- ATLOs contain germinal center B cells and both short-lived and long-lived plasma cells.
- ATLOs harbor an aberrant set of B-1 cells whose subtype is skewed toward B-1b cells.
- A fraction of ATLO B cells produce IgM or IgG.

American Heart Association.

Artery Tertiary Lymphoid Organs Control Multilayered Territorialized Atherosclerosis B-Cell Responses in Aged $ApoE^{-/-}$ Mice

Prasad Srikakulapu, Desheng Hu, Changjun Yin, Sarajo K. Mohanta, Sai Vineela Bontha, Li Peng, Michael Beer, Christian Weber, Coleen A. McNamara, Gianluca Grassia, Pasquale Maffia, Rudolf A. Manz and Andreas J.R. Habenicht

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/early/2016/04/21/ATVBAHA.115.306983
Free via Open Access

Data Supplement (unedited) at:
http://atvb.ahajournals.org/content/suppl/2016/04/20/ATVBAHA.115.306983.DC1.html
http://atvb.ahajournals.org/content/suppl/2016/04/20/ATVBAHA.115.306983.DC2.html

---

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Arteriosclerosis, Thrombosis, and Vascular Biology* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Arteriosclerosis, Thrombosis, and Vascular Biology* is online at:
http://atvb.ahajournals.org//subscriptions/

Aorta B Cell Proliferation and Maturation Pathways in ATLOs

Hypothetical choreography of ATLO B cell responses. Mature naïve B-2 or circulating B-1 cells enter ATLOs through high endothelial venules (HEVs). B-2 cells undergo T helper cell-dependent activation, proliferation, and maturation steps including somatic hypermutation and affinity maturation in germinal center reactions in response to arterial wall-derived autoantigen(s). The resulting high-affinity B-2 B cells either differentiate into memory B cells followed by isotype-switching and/or differentiation into plasma cells. A fraction of plasma cells expresses IL-10$^+$. B-1 cells are activated by inflammatory cytokines and - possibly in response to atherosclerosis autoantigens - undergo self-renewal to form IL-10$^-$ B-1a or IL-10$^+$ B-1b cells. T cell immune responses except T helper cells and T follicular helper cells or antigen-presenting cells are not depicted for ease of reading.

Materials and Methods

Mice. C57BL/6J WT and *ApoE*^{-/-} mice were purchased from the Jackson laboratory and housed in the animal facilities of University of Jena and Ludwig-Maximilians-University Munich Germany. Mice were maintained in a pathogen-free environment on a 12 hour light/dark cycle and fed standard chow. 78-80 weeks old male WT and *ApoE*^{-/-} mice were used. CD45.1/Ly5.1 mice were bred in the animal facility of the Leibniz-Institute for Age Research Jena, Germany. Animal procedures were conducted according to guidelines approved by the Animal Care and Use Committees.

Single cell preparation from aorta, spleen, RLN, blood, PerC, and BM. Single cell suspensions from aorta were prepared by enzymatic digestion as previously described[1] with further modifications as described[1, 2]. Briefly, WT and *ApoE*^{-/-} mice were euthanized and the aorta was perfused with 2 mM EDTA in PBS, PBS, and FACS buffer. Care was taken to remove adjacent adipose tissue and small paraaortic lymph nodes to avoid contamination with cells present in adjacent connective tissues including fat associated lymphoid clusters. Thoracic and abdominal aorta segments were harvested, cut into small pieces and digested in 2.5 ml of enzyme cocktail (450 U/ml collagenase type I, 125 U/ml collagenase type XI, 60 U/ml hyaluronidase and 60 U/ml DNase1) in Dulbecco's phosphate buffered saline (DPBS) containing 20 mM HEPES. Digestion was carried out at 37° C for 1 hour in a water bath with shaking. The cell suspension was filtered through a 70 µm cell strainer by mashing aorta pieces using a syringe plunger. The cell suspension was centrifuged and resuspended in FACS buffer. Cell suspensions from spleen and RLNs was prepared using a 70 µm cell strainer and mashing spleen and RLNs with a syringe plunger and dissolved in FACS buffer. For blood samples, blood (0.2 ml) was collected into 5 mM Na_2-EDTA in an Eppendorf tube, mixed, centrifuged, and washed. For BM, femurs and tibiae were prepared and the muscle was removed. After cutting the ends of femur and tibia, BM was flushed with FACS buffer, centrifuged, and washed. Spleen, BM, and blood samples were resuspended in erythrocyte lysis buffer and washed. After centrifugation, cells were washed and resuspended in FACS buffer and cells were counted. For PerC, peritoneal lavage was collected, washed, centrifuged, and resuspended in FACS buffer and cells were counted.

Detection and quantification of ATLOs. ATLOs were identified and their size was determined as previously described[1-3].

Flow cytometry. Fc receptors were blocked by pretreatment with purified anti-mouse CD16/32 mAb for 10 min at 4° C. Cells were incubated with fluorescent labelled antibodies for 25 min at 4° C and washed twice. Cells were incubated with streptavidin conjugate for 20 min at 4° C. After washing, 8-color FACS measurements were performed on FACSCanto II™ (BD Bioscience). Data were analysed using FlowJo (tree star). Labelled Abs that were used for FACS analyses: CD16/32 (93) (Fc-block); CD45-PerCpCy5.5 (30-F11); CD45.1-PerCpCy5.5 (A20); CD19-APC (ID3); CD19-eFluor 450 (ID3); CD23-FITC (B3B4), CD5-PE (53-7.3); CD43-Biotin (R2/60); CD11b-eFluor 450 (M1/70); CD38-PerCp eFluor-710 (90); PNA-FITC; GL7-Biotin (GL7); IgM-PECy7

(II/41); IgD-eFluor 450 (11-26c); IgG1-FITC (A85-1); IgA-PE (mA-6E1); IgE-Biotin (23G3) and Streptavidin-APC eFluor780; IL-10-PE(JES5-16E3); CD5-APC(53-7.3); TGF beta 1–PE; FasL-FITC; PD-L1-PE. All Abs are from eBioscience except PNA-FITC (Vector) and IgG1-FITC, CD138-APC (281-2) (BD Biosciences). All antisera were used at 1:200 dilutions for staining except PNA-FITC and GL7-Biotin at 1:300.

BrdU labeling and immunofluoroscence staining. *ApoE*^{-/-} mice (78-80 weeks) were fed with BrdU (1 mg/ml; Sigma-Aldrich) and 1% glucose (Merck) in drinking water for 2 weeks. Drinking water was protected from light and changed every 3 days[4]. After BrdU treatment, mice were euthanized and perfused with 3% paraformaldehyde (PFA) in 1x PBS at 120 Hg pressure for 30 min at RT. Fresh frozen tissue blocks were prepared for aorta and 10 μm sections were prepared for immunofluorescence analyses. Sections were washed with PBS for 10 min. To denature DNA, sections were incubated with 2 N HCl at 37° C for 15 min (only for BrdU staining) and washed 3 x 5 min with PBS. A blocking step was performed at RT for 1 hour in blocking solution (5% FBS, 1% BSA and 0.4% Triton X-100 in PBS). After blocking without washing, sections were incubated overnight with primary antibodies at 4° C. After primary antibody staining, sections were washed and incubated with fluorescent labelled secondary antibodies and DAPI at RT for 60 min. Sections were washed and mounted onto slides using fluorescence mounting medium (S3025, Dako). Immunofluorescence analyses were performed as described[1] using primary antibodies CD138 (1:50, R&D), BrdU (1:50, Oxford Biotechnology), CD45R/B220 (1:200, BD), IgG1-FITC (1:50, BD), IgM-FITC (1:50, BD), IgA-PE (1:50, eBioscience), IgE-Biotin (1:50, eBioscience), and corresponding fluorescent labelled secondary antibodies (1:300, all from Dianova).. For negative controls, stainings were performed without primary antibodies. Pictures were taken by LSM-510 META confocal scanning microscope (Zeiss) or SP5 (Leica) and analysed by Zeiss LSM image browser or Fiji image processing softwares.

MACS and ELISPOT assay. To identify constitutively IgM- and IgG-secreting B cells, ELISPOT assays were performed. Single cell suspensions of aortas were incubated with CD45 microbeads for 15 min at 4° C. Cells were washed, centrifuged and the pellet was resuspended in MACS running buffer. CD45^{+} cells were separated using the POSSEL-S program in Auto-MACS (Miltenyi Biotec) and purified >97%. MultiScreen$_{HTS}$ IP-Plates (MSIPS4510, Millipore) were used. Each well was rinsed according to manufacturer's protocol. After washing, wells were coated with unlabelled anti-mouse IgM or IgG antisera (10 μg/ml, Southern Biotech) and incubated overnight at 4° C. The antibody solution was decanted, wells were washed and blocked with RPMI-1640 + 10% FCS for 2 hours at 37° C. Cells were washed in cold complete RPMI-1640 medium to minimize background and a suspension of 10^6 cells/ml was prepared. After blocking, 0.25 x 10^6 cells from each tissue was added to the wells except for the aorta where total sample was added to the well and incubated overnight at 37° C. After 24 hours, cell culture dishes were decanted and washed with 0.1% Tween-20 in PBS. Biotin-labelled anti-mouse IgM or IgG antibody was (1:500, Southern Biotech) added to each well and incubated for 2 hours at 37° C. Plates were washed and streptavidin alkaline phosphatase (3:100, Abcam) was added to each well and incubated for 20-30 min at RT. Plates were washed, BCIP/NBT was added and incubated until spots became

visible. Pictures were taken under the dissection microscope (AxioCam dissection microscope, Zeiss) and spots were counted manually.

Cell stimulation for IL-10 staining. Cell stimulation and IL-10 staining were performed as described[5].

Adoptive B cell transfer experiments. B-2 B cell migration into ATLOs were determined following transfer of FACS-sorted B-2 B cells (>98% purity). 8 - 10 weeks old male CD45.1 (Ly5.1) mice (n=3-4 mice/sort) were used as donors. Spleens from 3-4 mice were pooled and single cells were prepared. Cells were stained with anti-CD19 APC, anti-CD43 PE, incubated, washed and DAPI⁻/CD19⁺/CD43⁻ B-2 B cells were sorted using a BD FACS Aria at a purity of >98% of total live cells. 30×10^6 cells per mouse were injected into aged $ApoE^{-/-}$ or WT mice by tail vein injection. After 36 hours, mice were analysed for migrated Ly5.1⁺ B-2 B cells in PerC, spleen, RLNs, BM, and aorta.

Laser capture microdissection (LCM) and microarray analysis. LCM and microarray analysis has been done as previously described[3, 6, 7]. Briefly, total aorta microarray analyses were performed on Affymetrix mouse whole genome 430 2.0 microarrays. Laser capture-derived RNA microarray analyses were performed with Affymetrix mouse whole genome 430A 2.0 microarrays. Signal intensities were calculated from the raw data and scaled to an array trimmed mean of 500. All further steps were performed using R and Bioconductor (R Development Core Team; Gentleman RC, *et al*. 2004). Logarithmic signals were normalized across arrays of every analysis using quantile normalization. Data were filtered prior to statistical analysis to remove genes with low expression or without variability between 2 groups: Probe sets were included if at least 2 arrays per group were called present (detection P≤0.05) and 2 or 3 arrays showed a log signal ≥ log2 (200) conditioned on a foldchange of at least log2 (2.0) between groups. To correct for lamina media RNA contamination in LCM experiments (error caused by nearby media tissue) on adventitia or plaque a correction algorhythm was performed: Up-regulated genes in WT adventitia, $ApoE^{-/-}$ adventitia, ATLO or plaque data were obtained as described[6]. Using the filtered data a one-factor variance analysis (ANOVA) or a two-sided T-test for two independent samples was applied and corrected with Benjamini-Hochberg correction for multiple testing.

Serum Antibodies detection by ELISAs. For the detection of total IgM and IgG, microtitre plates (Thermo Scientific, England, United Kingdom) were coated with serially diluted serum from aged ApoE⁻/⁻ and WT mice in PBS overnight at 4°C. The next day, plates were washed in PBS containing 0.05% tween and subsequently blocked using Animal-Free Blocker (Vector Laboratories, Peterborough, UK) for 1 hour at room temperature. Plates were subsequently washed and incubated with HRP conjugated rat anti-mouse IgM or IgG for 1 hour at room temperature. For the detection of total IgE a Ready-SET-Go Kit was used (Affymetrix, eBioscience). Briefly, plates were coated with 100 µl of anti-mouse IgE monoclonal antibody and incubated at 4°C degrees overnight. The next day, plates were washed in PBS containing 0.05% tween and incubated with 250 µl of blocking buffer at room temperature for 2 hours. After washing, plates were

incubated with serial dilutions of serum from aged ApoE$^{-/-}$ and WT mice for 4h at room temperature. The plates were then washed and incubated with 100 µl of biotin conjugated anti-mouse IgE monoclonal antibody at room temperature for 1 hour. After the washing the plates were finally incubated with HRP conjugated Streptavidin for 30 minutes at room temperature. After a final washing step, 100 µl of SureBlueTM TMB microwell peroxidase substrate (KPL, Gaithersburg, MD, USA) was added to each well. To stop the reaction, 50 µl of 10% hydrochloric acid was added to each well. Using the sunrise ELISA reader (Tecan, Mannedorf, Switzerland), plates were read at 450 nm with a reference of 630 nm.

Serum *anti*-malondialdehyde-oxidized-low density lipoprotein (anti-MDA-LDL) antibody detection by ELISA. For the detection of anti-MDA-LDL-specific antibodies, microtitre plates were coated with 100 µl of 12.5 µg/ml MDA-LDL (Nordic Biosite, Propellervagen, Sweden) in PBS overnight at 4° C. Next day, plates were washed, blocked and incubated for 4 hours at room temperature with serially diluted serum from aged *ApoE*$^{-/-}$ and aged WT mice. Plates were washed and incubated with HRP-conjugated goat anti-mouse IgG and IgM antisera for 1 hour at room temperature. After a final washing step, 100 µl of SureBlue^{TM} TMB microwell peroxidase substrate (KPL, Gaithersburg, MD, USA) was added to each well. To stop the reaction, 50 µl of 10% hydrochloric acid was added to each well. Using the Sunrise ELISA reader (Tecan, Mannedorf, Switzerland), plates were read at 450 nm with a reference of 630 nm.

Statistics. Data are expressed as means ± SEM. Data were analysed by two-tailed unpaired Student's t-test. One-way ANOVA with Bonferroni-Holm correction was done when multiple comparisons were made.

Supplemental References
1. Hu D, Mohanta S, Yin C, Weber C and Habenicht A. Preparation of Single Cell Suspensions from Mouse Aorta. Bio-protocol. 2016; in press.
2. Mohanta S, Yin C, Weber C, Hu D and Habenicht A. Aorta atherosclerosis lesion analysis in hyperlipidemic mice. Bio-Protocol. 2016; in press.
3. Hu D, Mohanta SK, Yin C, et al. Artery Tertiary Lymphoid Organs Control Aorta Immunity and Protect against Atherosclerosis via Vascular Smooth Muscle Cell Lymphotoxin beta Receptors. Immunity. 2015;42:1100-15.
4. Hoyer BF, Moser K, Hauser AE, Peddinghaus A, Voigt C, Eilat D, Radbruch A, Hiepe F and Manz RA. Short-lived plasmablasts and long-lived plasma cells contribute to chronic humoral autoimmunity in NZB/W mice. J Exp Med. 2004;199:1577-84.
5. Maseda D, Candando KM, Smith SH, Kalampokis I, Weaver CT, Plevy SE, Poe JC and Tedder TF. Peritoneal cavity regulatory B cells (B10 cells) modulate IFN-gamma+CD4+ T cell numbers during colitis development in mice. J Immunol. 2013;191:2780-95.
6. Beer M, Doepping S, Hildner M, Weber G, Grabner R, Hu D, Mohanta SK, Srikakulapu P, Weih F and Habenicht AJ. Laser-capture microdissection of hyperlipidemic/ApoE(-)/(-) mouse aorta atherosclerosis. Methods Mol Biol. 2011;755:417-28.

7. Yin C, Mohanta S, Ma Z, Weber C, Hu D, Weih F and Habenicht A. Generation of Aorta Transcript Atlases of Wild-Type and Apolipoprotein E-null Mice by Laser Capture Microdissection-Based mRNA Expression Microarrays. Methods Mol Biol. 2015;1339:297-308.

Supplement Material

Artery Tertiary Lymphoid Organs Control Multi-layered Territorialized Atherosclerosis B Cell Responses in Aged *ApoE*-/- Mice

Prasad Srikakulapu *et al*.

Supplemental Figures
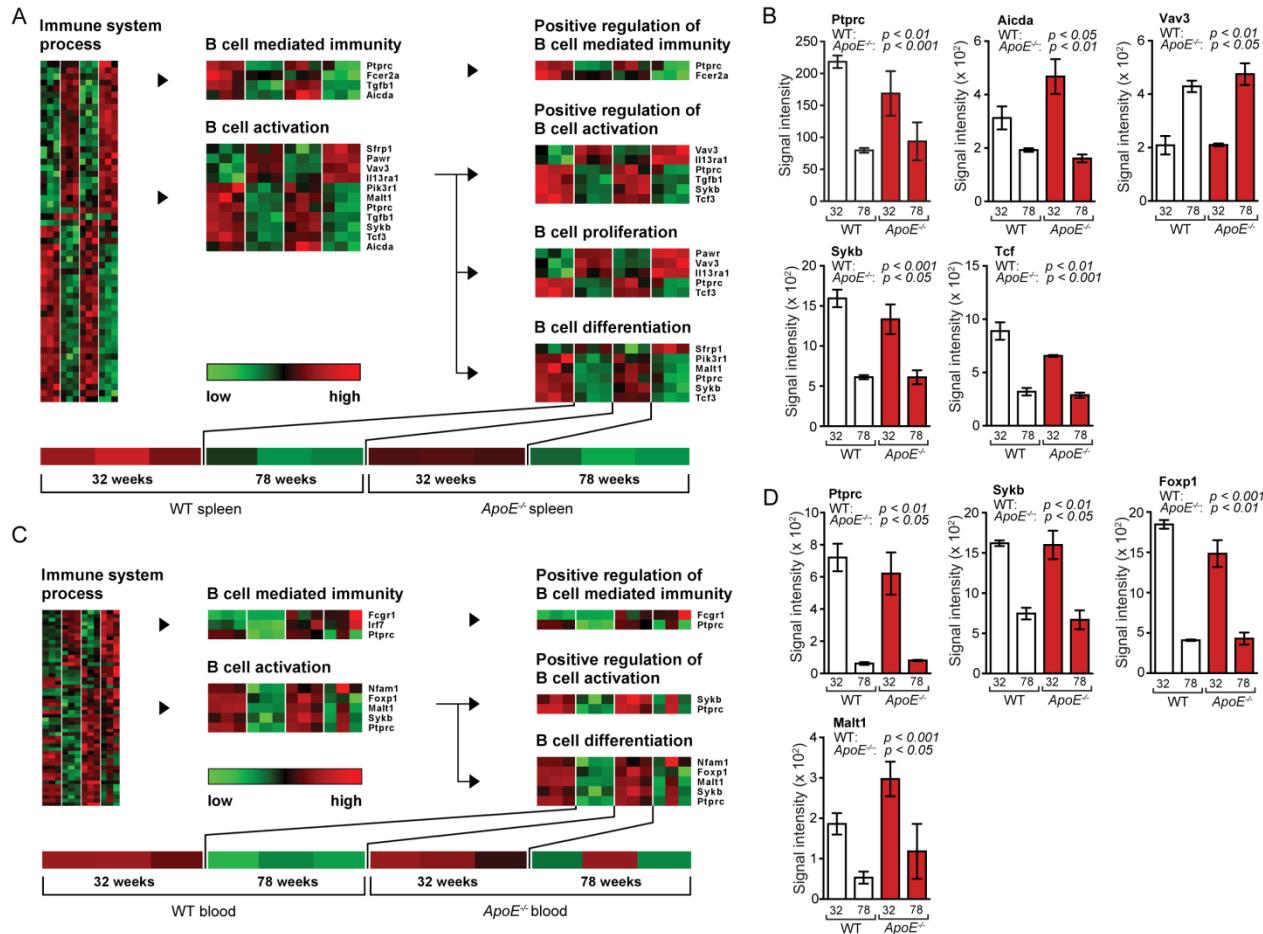
Figure SI: Age-associated B cell gene expression in spleen and blood of WT and *ApoE*-/- mice. Age-associated transcript profiles were obtained for total spleen and blood from 32 and 78 weeks old WT and *ApoE*-/- mice (n=3 mice per genotype per age group). Transcripts in GO terms Immune system process, B cell mediated immunity, B cell activation, positive regulation of B cell mediated immunity, positive regulation of B cell activation, B cell proliferation and B cell differentiation are displayed as heatmaps. Differential age- and genotype-associated gene expressions are shown as heatmaps or of selected genes, respectively in total spleen (**A, B**) and in blood (**C, D**) of WT and *ApoE*-/- mice. Results represent means±SEM. Analyses were performed using ANOVA with Benjamini-Hochberg correction. Absolute numbers of signal intensities and statistics are reported in supplementary Table S1.
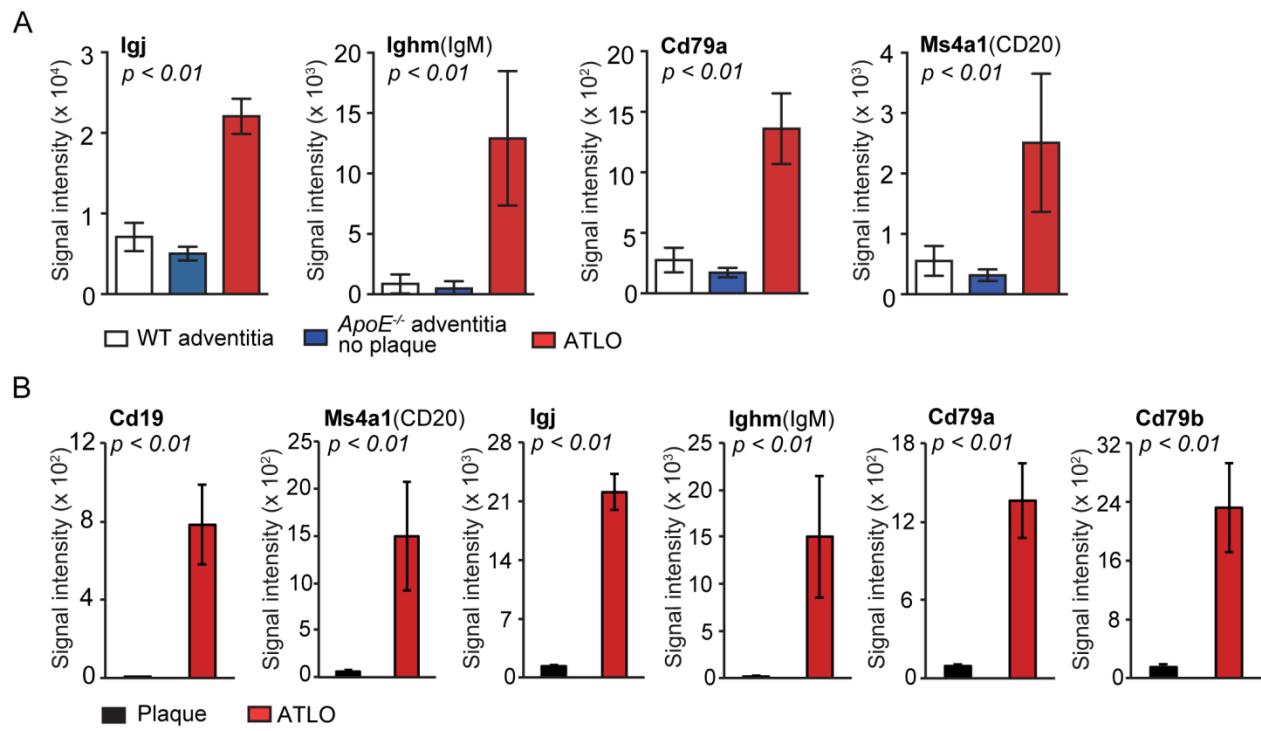
Figure SII: LCM microarray-based gene expression analysis of plaque versus ATLO. A) Differential expression of selected genes in adventitia cluster (WT n=3; *ApoE*^{-/-} n=4) and **B)** plaque/ATLO cluster (for plaque *ApoE*^{-/-} n=3; for ATLOs *ApoE*^{-/-} n=4). Results represent means ± SEM. Analyses were performed using ANOVA with Benjamini-Hochberg correction. Absolute numbers of signal intensities and statistics are reported in online supplement Table S1 and online methods.
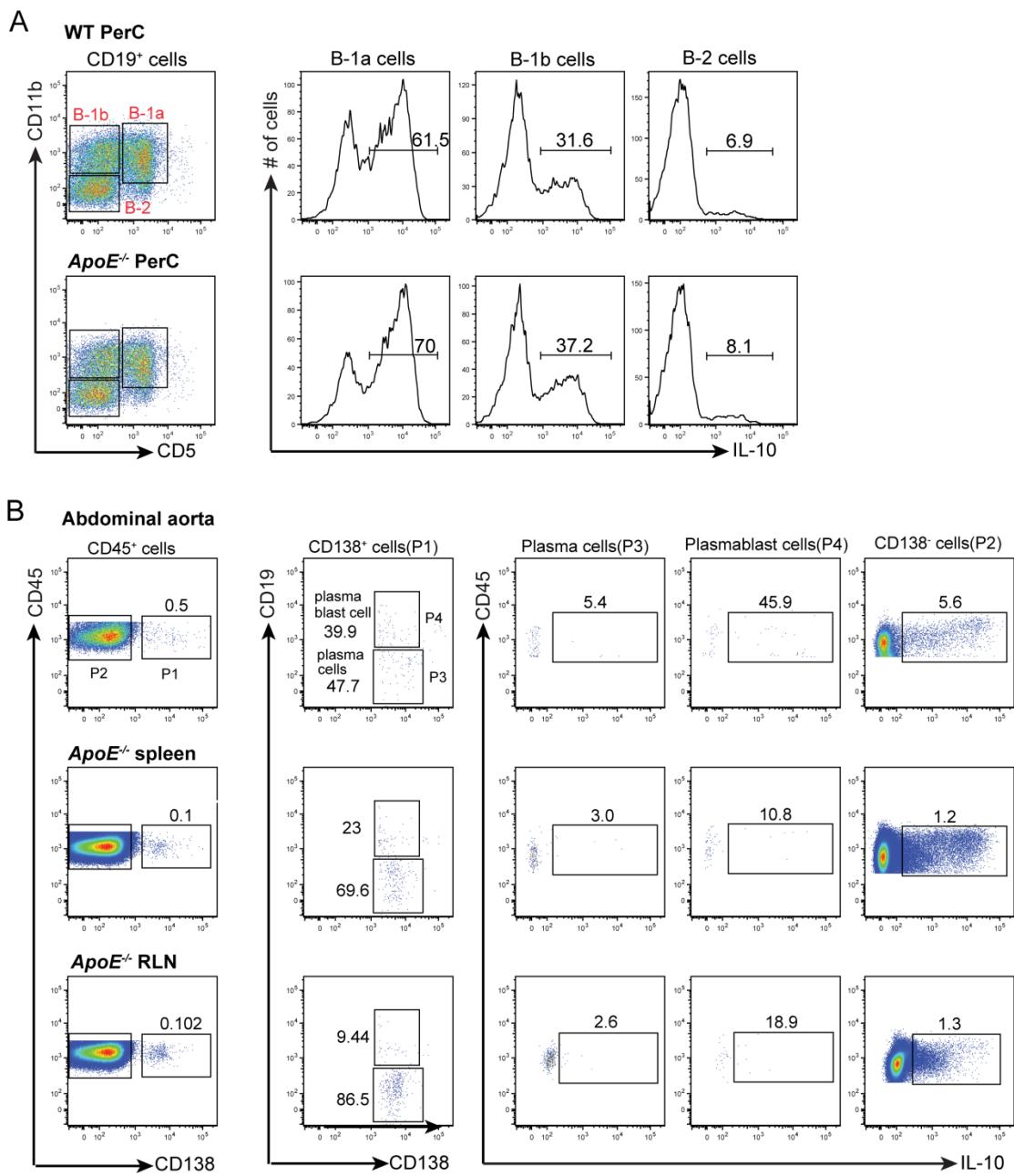
Figure SIII: IL-10 producing cells in perC, aorta, spleen, and RLN. Isolated cells from peritoneal cavity **A)** aorta, spleen and RLN **B)** of 80-85w old WT and *ApoE$^{-/-}$* mice (n=3 mice per genotype) were stimulated as decribed in methods. Cells were stained with different antibodies as indicated. **A)** B-1a, B-1b, B-2 cells; **B)** PCs, plasmablasts, and CD138^{-} cells were gated and examined for IL-10 expression. Numbers on FACS plots are frequencies of positive cells per gate.

Aorta B Cell Proliferation and Maturation Pathways in ATLOs
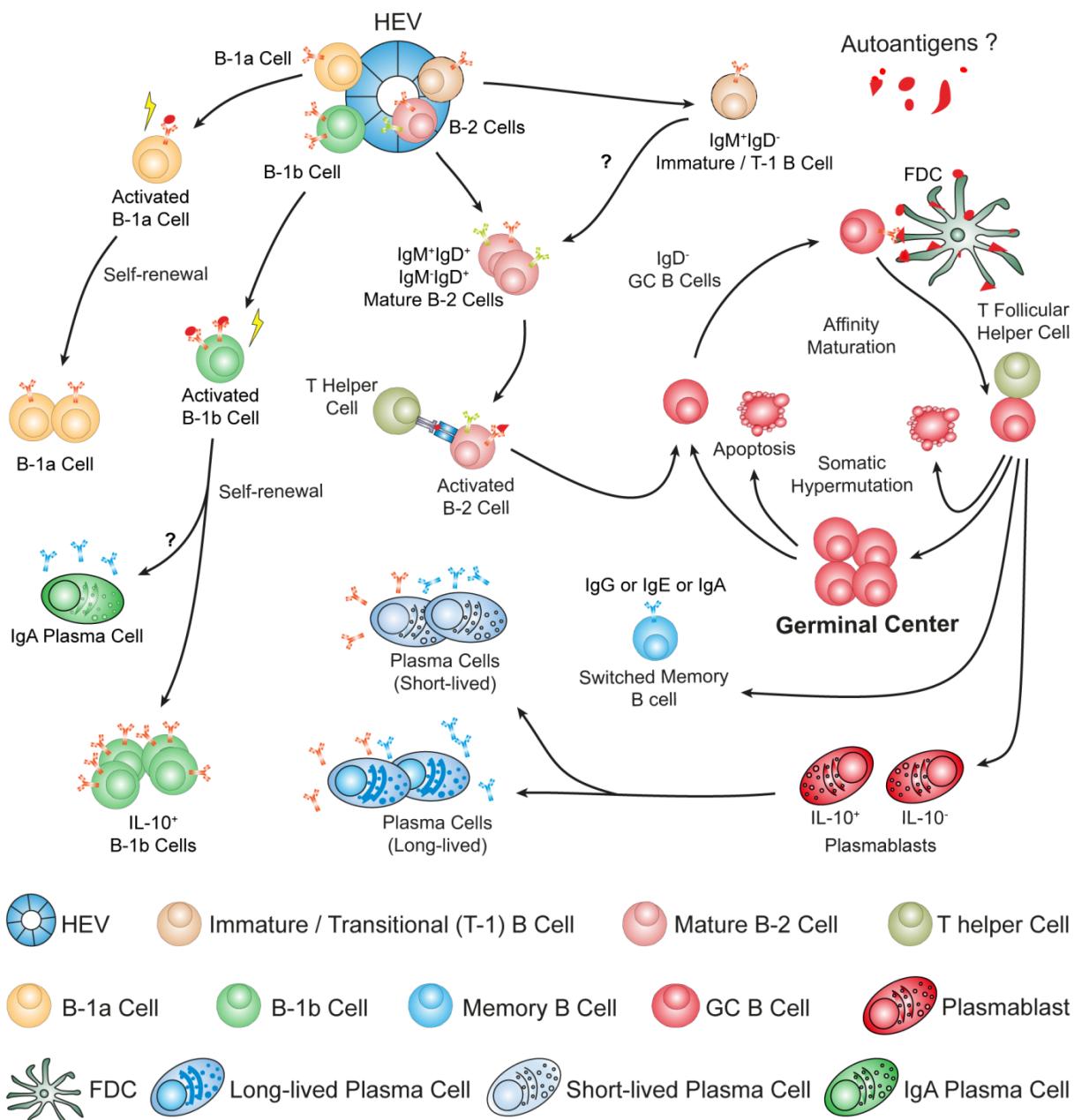
Figure SIV: Hypothetical choreography of ATLO B cell responses. Mature naïve B-2 or circulating B-1 cells enter ATLOs through high endothelial venules (HEVs). B-2 cells undergo T helper cell-dependent activation, proliferation, and maturation steps including somatic hypermutation and affinity maturation in germinal center reactions in response to arterial wall-derived autoantigen(s). The resulting high-affinity B-2 B cells either differentiate into memory B cells followed by isotype-switching and/or differentiation into plasma cells. A fraction of plasma cells expresses IL-10^{+}. B-1 cells are activated by inflammatory cytokines and - possibly in response to atherosclerosis autoantigens - undergo self-renewal to form IL-10^{-} B-1a or IL-10^{+} B-1b cells. T cell immune responses except T helper cells and T follicular helper cells or antigen-presenting cells are not depicted for ease of reading.

Supplementary table I. Transcript maps of aorta, spleen, blood, and RLNs of WT and *ApoE*$^{-/-}$ mice.

A. Differential expression of transcripts coding for B cell-immunity regulating genes in WT and *ApoE*$^{-/-}$ aorta, spleen, and blood

A1: Aging-aorta: Differential expression of transcripts coding B cell regulating genes in WT and *ApoE*$^{-/-}$ total aorta (Fig.1).

A) B cell mediated immunity

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT Aorta 6 weeks	Mean WT Aorta 32 weeks	Mean WT Aorta 78 weeks	Mean ApoE$^{-/-}$ Aorta 6 weeks	Mean ApoE$^{-/-}$ Aorta 32 weeks	Mean ApoE$^{-/-}$ Aorta 78 weeks	Fold change ApoE$^{-/-}$ Aorta 78 weeks vs. 6 weeks	p ANOVA
1425385_a_at	Ighm	immunoglobulin heavy constant mu	16019	88	124	211	108	189	14621	135.31	< 0.0001
1425324_x_at	Ighm	immunoglobulin heavy constant mu	16019	49	77	38	34	66	4275	126.80	0.0007
1427756_x_at	Ighm	immunoglobulin heavy constant mu	16019	82	67	73	51	91	4981	97.95	< 0.0001
1460188_at	Ptpn6	protein tyrosine phosphatase, non-receptor type 6	15170	37	51	198	46	567	1324	28.96	< 0.0001
1427329_a_at	Ighm	immunoglobulin heavy constant mu	16019	543	718	1806	648	992	17344	26.75	< 0.0001
1427351_s_at	Ighm	immunoglobulin heavy constant mu	16019	444	654	3608	658	1452	15930	24.20	0.001
1424302_at	Lilrb3	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 3	18733	86	45	107	56	506	1265	22.71	< 0.0001

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT Aorta 6 weeks	Mean WT Aorta 32 weeks	Mean WT Aorta 78 weeks	Mean ApoE$^{-/-}$ Aorta 6 weeks	Mean ApoE$^{-/-}$ Aorta 32 weeks	Mean ApoE$^{-/-}$ Aorta 78 weeks	Fold change ApoE$^{-/-}$ Aorta 78 weeks vs. 6 weeks	p ANOVA
1418340_at	Fcer1g	Fc receptor, IgE, high affinity I, gamma polypeptide	14127	507	690	889	382	2708	5707	14.95	< 0.0001
1425477_x_at	H2-Ab1	histocompatibility 2, class II antigen A, beta 1	14961	279	361	491	263	1416	3555	13.53	< 0.0001
1427870_x_at	Ighm	immunoglobulin heavy constant mu	16019	600	957	1072	623	660	7671	12.32	< 0.0001
1451721_a_at	H2-Ab1	histocompatibility 2, class II antigen A, beta 1	14961	457	616	941	514	2144	5102	9.93	< 0.0001
1448620_at	Fcgr3	Fc receptor, IgG, low affinity III	14131	324	357	399	369	1881	3536	9.58	< 0.0001
1450648_s_at	H2-Ab1	histocompatibility 2, class II antigen A, beta 1	14961	780	1005	1459	782	2234	6399	8.19	< 0.0001
1422124_a_at	Ptprc	protein tyrosine phosphatase, receptor type, C	19264	486	772	990	642	2454	5106	7.95	< 0.0001
1437025_at	Cd28	CD28 antigen	12487	44	83	77	36	151	265	7.29	0.02
1417063_at	C1qb	complement component 1, q subcomponent, beta polypeptide	12260	1347	1154	1506	1169	5621	8251	7.06	< 0.0001
1417876_at	Fcgr1	Fc receptor, IgG, high affinity I	14129	156	174	234	153	627	1066	6.96	< 0.0001
1425519_a_at	Cd74	CD74 antigen (invariant polypeptide of major histocompatibility complex,	16149	1634	2699	4537	1955	7675	11699	5.98	< 0.0001

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT Aorta 6 weeks	Mean WT Aorta 32 weeks	Mean WT Aorta 78 weeks	Mean ApoE$^{-/-}$ Aorta 6 weeks	Mean ApoE$^{-/-}$ Aorta 32 weeks	Mean ApoE$^{-/-}$ Aorta 78 weeks	Fold change ApoE$^{-/-}$ Aorta 78 weeks vs. 6 weeks	p ANOVA
		class II antigen-associated)									
1422527_at	H2-DMa	histocompatibility 2, class II, locus DMa	14998	230	277	449	231	546	1370	5.92	< 0.0001
1418133_at	Bcl3	B cell leukemia/lymphoma 3	12051	42	138	136	80	335	461	5.73	0.0006
1435477_s_at	Fcgr2b	Fc receptor, IgG, low affinity IIb	14130	736	720	1048	705	2176	3922	5.56	< 0.0001
1451941_a_at	Fcgr2b	Fc receptor, IgG, low affinity IIb	14130	549	458	690	506	1472	2594	5.13	< 0.0001
1418021_at	C4b	complement component 4B (Childo blood group)	12268	1511	2739	2438	1737	6446	8292	4.77	< 0.0001
1437726_x_at	C1qb	complement component 1, q subcomponent, beta polypeptide	12260	1568	1745	1897	1774	7646	8089	4.56	< 0.0001
1417381_at	C1qa	complement component 1, q subcomponent, alpha polypeptide	12259	1952	1766	1881	1925	6761	8373	4.35	< 0.0001
1455332_x_at	Fcgr2b	Fc receptor, IgG, low affinity IIb	14130	254	277	421	334	907	1444	4.32	< 0.0001
1423954_at	C3	complement component 3	12266	1929	4798	3722	2416	8194	10023	4.15	< 0.0001
1420653_at	Tgfb1	transforming groWTh factor, beta 1	21803	516	538	551	440	1292	1670	3.79	< 0.0001

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT Aorta 6 weeks	Mean WT Aorta 32 weeks	Mean WT Aorta 78 weeks	Mean ApoE$^{-/-}$ Aorta 6 weeks	Mean ApoE$^{-/-}$ Aorta 32 weeks	Mean ApoE$^{-/-}$ Aorta 78 weeks	Fold change ApoE$^{-/-}$ Aorta 78 weeks vs. 6 weeks	p ANOVA
1418110_a_at	Inpp5d	inositol polyphosphate-5-phosphatase D	16331	345	428	498	344	752	1281	3.72	< 0.0001
1422122_at	Fcer2a	Fc receptor, IgE, low affinity II, alpha polypeptide	14128	140	73	232	93	112	342	3.67	0.02
1449473_s_at	Cd40	CD40 antigen	21939	84	45	100	68	145	230	3.39	0.03
1417597_at	Cd28	CD28 antigen	12487	81	91	65	83	114	282	3.39	0.0001
1434366_x_at	C1qb	complement component 1, q subcomponent, beta polypeptide	12260	1499	1543	1546	1518	5822	4861	3.20	< 0.0001
1456694_x_at	Ptpn6	protein tyrosine phosphatase, non-receptor type 6	15170	449	549	568	367	660	1075	2.93	0.001
1452535_at	Ighm	immunoglobulin heavy constant mu	16019	171	163	236	175	123	494	2.82	0.001
1436625_at	Fcgr1	Fc receptor, IgG, high affinity I	14129	150	172	282	182	348	484	2.65	0.001
1417244_a_at	Irf7	interferon regulatory factor 7	54123	253	302	393	266	425	633	2.38	0.0004
1423543_at	Swap70	SWA-70 protein	20947	678	412	468	498	490	977	1.96	0.0008
1460415_a_at	Cd40	CD40 antigen	21939	95	54	83	119	130	222	1.88	0.01
1424195_a_at	Inpp5d	inositol polyphosphate-5-phosphatase D	16331	277	295	192	234	344	410	1.75	0.0001

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT Aorta 6 weeks	Mean WT Aorta 32 weeks	Mean WT Aorta 78 weeks	Mean ApoE$^{-/-}$ Aorta 6 weeks	Mean ApoE$^{-/-}$ Aorta 32 weeks	Mean ApoE$^{-/-}$ Aorta 78 weeks	Fold change ApoE$^{-/-}$ Aorta 78 weeks vs. 6 weeks	p ANOVA
1457664_x_at	C2	complement component 2 (within H-2S)	12263	357	255	619	365	420	573	1.57	0.002
1437270_a_at	Clcf1	cardiotrophin-like cytokine factor 1	56708	122	162	123	194	244	248	1.28	0.001
1421818_at	Bcl6	B cell leukemia/lymphoma 6	12053	1904	700	919	1243	862	1208	-1.03	0.007
1450381_a_at	Bcl6	B cell leukemia/lymphoma 6	12053	843	384	390	624	366	434	-1.44	0.0005

B) B cell activation

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT Aorta 6 weeks	Mean WT Aorta 32 weeks	Mean WT Aorta 78 weeks	Mean ApoE$^{-/-}$ Aorta 6 weeks	Mean ApoE$^{-/-}$ Aorta 32 weeks	Mean ApoE$^{-/-}$ Aorta 78 weeks	Fold change ApoE$^{-/-}$ Aorta 78 weeks vs. 6 weeks	p ANOVA
1425385_a_at	Ighm	immunoglobulin heavy constant mu	16019	88	124	211	108	189	14621	135.31	< 0.0001
1425324_x_at	Ighm	immunoglobulin heavy constant mu	16019	49	77	38	34	66	4275	126.80	0.0007
1427756_x_at	Ighm	immunoglobulin heavy constant mu	16019	82	67	73	51	91	4981	97.95	< 0.0001

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT Aorta 6 weeks	Mean WT Aorta 32 weeks	Mean WT Aorta 78 weeks	Mean ApoE$^{-/-}$ Aorta 6 weeks	Mean ApoE$^{-/-}$ Aorta 32 weeks	Mean ApoE$^{-/-}$ Aorta 78 weeks	Fold change ApoE$^{-/-}$ Aorta 78 weeks vs. 6 weeks	p ANOVA
1448576_at	Il7r	interleukin 7 receptor	16197	9	10	17	21	471	938	45.04	< 0.0001
1460188_at	Ptpn6	protein tyrosine phosphatase, non-receptor type 6	15170	37	51	198	46	567	1324	28.96	< 0.0001
1436312_at	Ikzf1	IKAROS family zinc finger 1	22778	40	43	212	35	338	948	27.12	< 0.0001
1427329_a_at	Ighm	immunoglobulin heavy constant mu	16019	543	718	1806	648	992	17344	26.75	< 0.0001
1449858_at	Cd86	CD86 antigen	12524	15	8	21	11	107	296	25.86	0.001
1450297_at	Il6	interleukin 6	16193	21	10	57	21	290	523	24.46	0.0001
1427351_s_at	Ighm	immunoglobulin heavy constant mu	16019	444	654	3608	658	1452	15930	24.20	0.001
1448575_at	Il7r	interleukin 7 receptor	16197	106	150	203	136	1187	3130	22.99	< 0.0001
1451780_at	Blnk	B cell linker	17060	154	188	302	172	1038	2733	15.93	< 0.0001
1423478_at	Prkcb	protein kinase C, beta	18751	16	30	23	23	185	358	15.24	< 0.0001
1423182_at	Tnfrsf13b	tumor necrosis factor receptor superfamily, member 13b	57916	39	53	123	46	162	647	14.17	< 0.0001
1425477_x_at	H2-Ab1	histocompatibility 2, class II antigen A, beta 1	14961	279	361	491	263	1416	3555	13.53	< 0.0001
1427870_x_at	Ighm	immunoglobulin heavy constant mu	16019	600	957	1072	623	660	7671	12.32	< 0.0001

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT Aorta 6 weeks	Mean WT Aorta 32 weeks	Mean WT Aorta 78 weeks	Mean ApoE$^{-/-}$ Aorta 6 weeks	Mean ApoE$^{-/-}$ Aorta 32 weeks	Mean ApoE$^{-/-}$ Aorta 78 weeks	Fold change ApoE$^{-/-}$ Aorta 78 weeks vs. 6 weeks	p ANOVA
1450912_at	Ms4a1	membrane-spanning 4-domains, subfamily A, member 1	12482	29	44	95	48	52	564	11.67	0.002
1428787_at	Nckap1l	NCK associated protein 1 like	105855	130	86	249	159	779	1589	10.00	< 0.0001
1451721_a_at	H2-Ab1	histocompatibility 2, class II antigen A, beta 1	14961	457	616	941	514	2144	5102	9.93	< 0.0001
1418830_at	Cd79a	CD79A antigen (immunoglobulin-associated alpha)	12518	112	143	394	127	150	1235	9.73	0.0001
1426169_a_at	Lat2	linker for activation of T cells family, member 2	56743	137	187	162	115	529	1048	9.14	< 0.0001
1450648_s_at	H2-Ab1	histocompatibility 2, class II antigen A, beta 1	14961	780	1005	1459	782	2234	6399	8.19	< 0.0001
1460419_a_at	Prkcb	protein kinase C, beta	18751	250	359	588	265	1076	2126	8.01	< 0.0001
1422124_a_at	Ptprc	protein tyrosine phosphatase, receptor type, C	19264	486	772	990	642	2454	5106	7.95	< 0.0001
1436649_at	Ikzf3	IKAROS family zinc finger 3	22780	59	95	120	86	115	650	7.55	0.0002
1437025_at	Cd28	CD28 antigen	12487	44	83	77	36	151	265	7.29	0.02
1416296_at	Il2rg	interleukin 2 receptor, gamma chain	16186	281	418	374	235	769	1613	6.86	0.003

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT Aorta 6 weeks	Mean WT Aorta 32 weeks	Mean WT Aorta 78 weeks	Mean ApoE$^{-/-}$ Aorta 6 weeks	Mean ApoE$^{-/-}$ Aorta 32 weeks	Mean ApoE$^{-/-}$ Aorta 78 weeks	Fold change ApoE$^{-/-}$ Aorta 78 weeks vs. 6 weeks	p ANOVA
1418261_at	Sykb	spleen tyrosine kinase	20963	311	375	517	281	875	1860	6.63	< 0.0001
1423226_at	Ms4a1	membrane-spanning 4-domains, subfamily A, member 1	12482	225	213	388	241	240	1570	6.51	< 0.0001
1422631_at	Ahr	aryl-hydrocarbon receptor	11622	227	284	231	185	675	1161	6.29	< 0.0001
1425519_a_at	Cd74	CD74 antigen (invariant polypeptide of major histocompatibility complex, class II antigen-associated)	16149	1634	2699	4537	1955	7675	11699	5.98	< 0.0001
1428786_at	Nckap1l	NCK associated protein 1 like	105855	283	375	583	364	896	2157	5.92	< 0.0001
1437356_at	Gpr183	G protein-coupled receptor 183	321019	103	133	222	107	250	617	5.75	< 0.0001
1418133_at	Bcl3	B cell leukemia/lymphoma 3	12051	42	138	136	80	335	461	5.73	0.0006
1435477_s_at	Fcgr2b	Fc receptor, IgG, low affinity IIb	14130	736	720	1048	705	2176	3922	5.56	< 0.0001
1416295_a_at	Il2rg	interleukin 2 receptor, gamma chain	16186	369	525	720	475	1479	2625	5.53	< 0.0001
1436861_at	Il7	interleukin 7	16196	73	104	123	46	135	240	5.23	0.0001
1451941_a_at	Fcgr2b	Fc receptor, IgG, low affinity IIb	14130	549	458	690	506	1472	2594	5.13	< 0.0001
1418262_at	Sykb	spleen tyrosine kinase	20963	149	133	185	133	404	664	4.98	< 0.0001

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT Aorta 6 weeks	Mean WT Aorta 32 weeks	Mean WT Aorta 78 weeks	Mean ApoE$^{-/-}$ Aorta 6 weeks	Mean ApoE$^{-/-}$ Aorta 32 weeks	Mean ApoE$^{-/-}$ Aorta 78 weeks	Fold change ApoE$^{-/-}$ Aorta 78 weeks vs. 6 weeks	p ANOVA
1455332_x_at	Fcgr2b	Fc receptor, IgG, low affinity IIb	14130	254	277	421	334	907	1444	4.32	< 0.0001
1427007_at	Sash3	SAM and SH3 domain containing 3	74131	288	345	516	338	740	1354	4.00	< 0.0001
1456328_at	Bank1	B cell scaffold protein with ankyrin repeats 1	242248	277	356	517	297	349	1176	3.96	0.0007
1420653_at	Tgfb1	transforming groWTh factor, beta 1	21803	516	538	551	440	1292	1670	3.79	< 0.0001
1418110_a_at	Inpp5d	inositol polyphosphate-5-phosphatase D	16331	345	428	498	344	752	1281	3.72	< 0.0001
1450136_at	Cd38	CD38 antigen	12494	141	123	131	177	578	644	3.64	< 0.0001
1433741_at	Cd38	CD38 antigen	12494	414	530	662	531	1318	1894	3.57	< 0.0001
1455656_at	Btla	B and T lymphocyte associated	208154	218	287	373	282	342	1006	3.57	0.0002
1449473_s_at	Cd40	CD40 antigen	21939	84	45	100	68	145	230	3.39	0.03
1417597_at	Cd28	CD28 antigen	12487	81	91	65	83	114	282	3.39	0.0001
1425289_a_at	Cr2	complement receptor 2	12902	132	161	241	118	135	396	3.35	0.02
1422003_at	Cxcr5	chemokine (C-X-C motif) receptor 5	12145	86	94	179	101	90	326	3.23	0.0003
1456694_x_at	Ptpn6	protein tyrosine phosphatase, non-	15170	449	549	568	367	660	1075	2.93	0.001

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT Aorta 6 weeks	Mean WT Aorta 32 weeks	Mean WT Aorta 78 weeks	Mean ApoE$^{-/-}$ Aorta 6 weeks	Mean ApoE$^{-/-}$ Aorta 32 weeks	Mean ApoE$^{-/-}$ Aorta 78 weeks	Fold change ApoE$^{-/-}$ Aorta 78 weeks vs. 6 weeks	p ANOVA
		receptor type 6									
1420425_at	Prdm1	PR domain containing 1, with ZNF domain	12142	144	169	175	159	193	463	2.92	0.002
1420404_at	Cd86	CD86 antigen	12524	160	152	167	141	232	402	2.85	0.0006
1452535_at	Ighm	immunoglobulin heavy constant mu	16019	171	163	236	175	123	494	2.82	0.001
1421457_a_at	Samsn1	SAM domain, SH3 domain and nuclear localization signals, 1	67742	86	122	145	102	185	283	2.76	0.0001
1419307_at	Tnfrsf13c	tumor necrosis factor receptor superfamily, member 13c	72049	141	192	284	171	183	472	2.76	0.005
1453281_at	Pik3cd	phosphatidylinositol 3-kinase catalytic delta polypeptide	18707	228	201	252	235	350	630	2.69	0.0005
1423319_at	Hhex	hematopoietically expressed homeobox	15242	379	409	524	346	644	902	2.61	0.0001
1460255_at	Tnfsf13b	tumor necrosis factor (ligand) superfamily, member 13b	24099	340	449	496	381	613	967	2.54	< 0.0001
1417122_at	Vav3	vav 3 oncogene	57257	150	247	205	178	308	447	2.51	0.001
1427164_at	Il13ra1	interleukin 13 receptor, alpha 1	16164	234	144	152	186	325	463	2.49	< 0.0001

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT Aorta 6 weeks	Mean WT Aorta 32 weeks	Mean WT Aorta 78 weeks	Mean ApoE$^{-/-}$ Aorta 6 weeks	Mean ApoE$^{-/-}$ Aorta 32 weeks	Mean ApoE$^{-/-}$ Aorta 78 weeks	Fold change ApoE$^{-/-}$ Aorta 78 weeks vs. 6 weeks	p ANOVA
1439205_at	Nfatc2	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 2	18019	218	264	301	221	364	538	2.43	0.0006
1433699_at	Tnfaip3	tumor necrosis factor, alpha-induced protein 3	21929	569	683	845	643	1032	1441	2.24	0.0001
1448600_s_at	Vav3	vav 3 oncogene	57257	212	366	305	250	365	542	2.17	0.0006
1423543_at	Swap70	SWA-70 protein	20947	678	412	468	498	490	977	1.96	0.0008
1460415_a_at	Cd40	CD40 antigen	21939	95	54	83	119	130	222	1.88	0.01
1454783_at	Il13ra1	interleukin 13 receptor, alpha 1	16164	674	636	504	582	1076	1069	1.84	< 0.0001
1450829_at	Tnfaip3	tumor necrosis factor, alpha-induced protein 3	21929	50	77	119	133	144	239	1.80	0.01
1424195_a_at	Inpp5d	inositol polyphosphate-5-phosphatase D	16331	277	295	192	234	344	410	1.75	0.0001
1437270_a_at	Clcf1	cardiotrophin-like cytokine factor 1	56708	122	162	123	194	244	248	1.28	0.001
1421818_at	Bcl6	B cell leukemia/lymphoma 6	12053	1904	700	919	1243	862	1208	-1.03	0.007
1448395_at	Sfrp1	secreted frizzled-related protein 1	20377	1584	999	626	1162	1758	870	-1.33	0.0003
1421857_at	Adam17	a disintegrin and metallopeptidase	11491	849	443	318	746	452	556	-1.34	0.0003

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT Aorta 6 weeks	Mean WT Aorta 32 weeks	Mean WT Aorta 78 weeks	Mean ApoE$^{-/-}$ Aorta 6 weeks	Mean ApoE$^{-/-}$ Aorta 32 weeks	Mean ApoE$^{-/-}$ Aorta 78 weeks	Fold change ApoE$^{-/-}$ Aorta 78 weeks vs. 6 weeks	p ANOVA
		domain 17									
1456429_at	Malt1	mucosa associated lymphoid tissue lymphoma translocation gene 1	240354	466	236	169	385	312	278	-1.39	0.02
1431061_s_at	Peli1	pellino 1	67245	503	198	269	387	222	270	-1.43	0.002
1450381_a_at	Bcl6	B cell leukemia/lymphoma 6	12053	843	384	390	624	366	434	-1.44	0.0005
1416594_at	Sfrp1	secreted frizzled-related protein 1	20377	476	46	81	379	384	230	-1.64	< 0.0001
1422102_a_at	Stat5b	signal transducer and activator of transcription 5B	20851	326	292	265	404	190	213	-1.89	0.002
1434572_at	Hdac9	histone deacetylase 9	79221	1063	818	854	827	532	419	-1.98	0.0001
1442827_at	Tlr4	toll-like receptor 4	21898	413	183	137	320	208	142	-2.26	0.0009
1421028_a_at	Mef2c	myocyte enhancer factor 2C	17260	1504	712	697	1362	767	561	-2.43	< 0.0001
1421027_a_at	Mef2c	myocyte enhancer factor 2C	17260	2673	1597	972	2514	1694	854	-2.94	< 0.0001
1421306_a_at	Hdac9	histone deacetylase 9	79221	305	172	99	215	135	54	-3.94	0.0005

C) Positive regulation of B cell mediated immunity

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT Aorta 6 weeks	Mean WT Aorta 32 weeks	Mean WT Aorta 78 weeks	Mean ApoE$^{-/-}$ Aorta 6 weeks	Mean ApoE$^{-/-}$ Aorta 32 weeks	Mean ApoE$^{-/-}$ Aorta 78 weeks	Fold change ApoE$^{-/-}$ Aorta 78 weeks vs. 6 weeks	p ANOVA
1418340_at	Fcer1g	Fc receptor, IgE, high affinity I, gamma polypeptide	14127	507	690	889	382	2708	5707	14.95	< 0.0001
1448620_at	Fcgr3	Fc receptor, IgG, low affinity III	14131	324	357	399	369	1881	3536	9.58	< 0.0001
1422124_a_at	Ptprc	protein tyrosine phosphatase, receptor type, C	19264	486	772	990	642	2454	5106	7.95	< 0.0001
1417876_at	Fcgr1	Fc receptor, IgG, high affinity I	14129	156	174	234	153	627	1066	6.96	< 0.0001
1423954_at	C3	complement component 3	12266	1929	4798	3722	2416	8194	10023	4.15	< 0.0001
1422122_at	Fcer2a	Fc receptor, IgE, low affinity II, alpha polypeptide	14128	140	73	232	93	112	342	3.67	0.02
1436625_at	Fcgr1	Fc receptor, IgG, high affinity I	14129	150	172	282	182	348	484	2.65	0.001

D) Positive regulation of B cell activation

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT Aorta 6 weeks	Mean WT Aorta 32 weeks	Mean WT Aorta 78 weeks	Mean ApoE$^{-/-}$ Aorta 6 weeks	Mean ApoE$^{-/-}$ Aorta 32 weeks	Mean ApoE$^{-/-}$ Aorta 78 weeks	Fold change ApoE$^{-/-}$ Aorta 78 weeks vs. 6 weeks	p ANOVA
1425385_a_at	Ighm	immunoglobulin heavy constant mu	16019	88	124	211	108	189	14621	135.31	< 0.0001
1425324_x_at	Ighm	immunoglobulin heavy constant mu	16019	49	77	38	34	66	4275	126.80	0.0007
1427756_x_at	Ighm	immunoglobulin heavy constant mu	16019	82	67	73	51	91	4981	97.95	< 0.0001
1427329_a_at	Ighm	immunoglobulin heavy constant mu	16019	543	718	1806	648	992	17344	26.75	< 0.0001
1450297_at	Il6	interleukin 6	16193	21	10	57	21	290	523	24.46	0.0001
1427351_s_at	Ighm	immunoglobulin heavy constant mu	16019	444	654	3608	658	1452	15930	24.20	0.001
1427870_x_at	Ighm	immunoglobulin heavy constant mu	16019	600	957	1072	623	660	7671	12.32	< 0.0001
1428787_at	Nckap1l	NCK associated protein 1 like	105855	130	86	249	159	779	1589	10.00	< 0.0001
1422124_a_at	Ptprc	protein tyrosine phosphatase, receptor type, C	19264	486	772	990	642	2454	5106	7.95	< 0.0001
1437025_at	Cd28	CD28 antigen	12487	44	83	77	36	151	265	7.29	0.02
1416296_at	Il2rg	interleukin 2 receptor, gamma chain	16186	281	418	374	235	769	1613	6.86	0.003
1418261_at	Sykb	spleen tyrosine kinase	20963	311	375	517	281	875	1860	6.63	< 0.0001

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT Aorta 6 weeks	Mean WT Aorta 32 weeks	Mean WT Aorta 78 weeks	Mean ApoE$^{-/-}$ Aorta 6 weeks	Mean ApoE$^{-/-}$ Aorta 32 weeks	Mean ApoE$^{-/-}$ Aorta 78 weeks	Fold change ApoE$^{-/-}$ Aorta 78 weeks vs. 6 weeks	p ANOVA
1425519_a_at	Cd74	CD74 antigen (invariant polypeptide of major histocompatibility complex, class II antigen-associated)	16149	1634	2699	4537	1955	7675	11699	5.98	< 0.0001
1428786_at	Nckap1l	NCK associated protein 1 like	105855	283	375	583	364	896	2157	5.92	< 0.0001
1416295_a_at	Il2rg	interleukin 2 receptor, gamma chain	16186	369	525	720	475	1479	2625	5.53	< 0.0001
1436861_at	Il7	interleukin 7	16196	73	104	123	46	135	240	5.23	0.0001
1418262_at	Sykb	spleen tyrosine kinase	20963	149	133	185	133	404	664	4.98	< 0.0001
1427007_at	Sash3	SAM and SH3 domain containing 3	74131	288	345	516	338	740	1354	4.00	< 0.0001
1420653_at	Tgfb1	transforming groWTh factor, beta 1	21803	516	538	551	440	1292	1670	3.79	< 0.0001
1418110_a_at	Inpp5d	inositol polyphosphate-5-phosphatase D	16331	345	428	498	344	752	1281	3.72	< 0.0001
1450136_at	Cd38	CD38 antigen	12494	141	123	131	177	578	644	3.64	< 0.0001
1433741_at	Cd38	CD38 antigen	12494	414	530	662	531	1318	1894	3.57	< 0.0001
1449473_s_at	Cd40	CD40 antigen	21939	84	45	100	68	145	230	3.39	0.03
1417597_at	Cd28	CD28 antigen	12487	81	91	65	83	114	282	3.39	0.0001

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT Aorta 6 weeks	Mean WT Aorta 32 weeks	Mean WT Aorta 78 weeks	Mean ApoE$^{-/-}$ Aorta 6 weeks	Mean ApoE$^{-/-}$ Aorta 32 weeks	Mean ApoE$^{-/-}$ Aorta 78 weeks	Fold change ApoE$^{-/-}$ Aorta 78 weeks vs. 6 weeks	p ANOVA
1420425_at	Prdm1	PR domain containing 1, with ZNF domain	12142	144	169	175	159	193	463	2.92	0.002
1452535_at	Ighm	immunoglobulin heavy constant mu	16019	171	163	236	175	123	494	2.82	0.001
1419307_at	Tnfrsf13c	tumor necrosis factor receptor superfamily, member 13c	72049	141	192	284	171	183	472	2.76	0.005
1460255_at	Tnfsf13b	tumor necrosis factor (ligand) superfamily, member 13b	24099	340	449	496	381	613	967	2.54	< 0.0001
1417122_at	Vav3	vav 3 oncogene	57257	150	247	205	178	308	447	2.51	0.001
1427164_at	Il13ra1	interleukin 13 receptor, alpha 1	16164	234	144	152	186	325	463	2.49	< 0.0001
1439205_at	Nfatc2	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 2	18019	218	264	301	221	364	538	2.43	0.0006
1448600_s_at	Vav3	vav 3 oncogene	57257	212	366	305	250	365	542	2.17	0.0006
1460415_a_at	Cd40	CD40 antigen	21939	95	54	83	119	130	222	1.88	0.01
1454783_at	Il13ra1	interleukin 13 receptor, alpha 1	16164	674	636	504	582	1076	1069	1.84	< 0.0001
1424195_a_at	Inpp5d	inositol polyphosphate-5-phosphatase D	16331	277	295	192	234	344	410	1.75	0.0001

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT Aorta 6 weeks	Mean WT Aorta 32 weeks	Mean WT Aorta 78 weeks	Mean ApoE$^{-/-}$ Aorta 6 weeks	Mean ApoE$^{-/-}$ Aorta 32 weeks	Mean ApoE$^{-/-}$ Aorta 78 weeks	Fold change ApoE$^{-/-}$ Aorta 78 weeks vs. 6 weeks	p ANOVA
1437270_a_at	Clcf1	cardiotrophin-like cytokine factor 1	56708	122	162	123	194	244	248	1.28	0.001
1421818_at	Bcl6	B cell leukemia/lymphoma 6	12053	1904	700	919	1243	862	1208	-1.03	0.007
1431061_s_at	Peli1	pellino 1	67245	503	198	269	387	222	270	-1.43	0.002
1450381_a_at	Bcl6	B cell leukemia/lymphoma 6	12053	843	384	390	624	366	434	-1.44	0.0005
1422102_a_at	Stat5b	signal transducer and activator of transcription 5B	20851	326	292	265	404	190	213	-1.89	0.002
1442827_at	Tlr4	toll-like receptor 4	21898	413	183	137	320	208	142	-2.26	0.0009

E) B cell proliferation

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT Aorta 6 weeks	Mean WT Aorta 32 weeks	Mean WT Aorta 78 weeks	Mean ApoE$^{-/-}$ Aorta 6 weeks	Mean ApoE$^{-/-}$ Aorta 32 weeks	Mean ApoE$^{-/-}$ Aorta 78 weeks	Fold change ApoE$^{-/-}$ Aorta 78 weeks vs. 6 weeks	p ANOVA
1425385_a_at	Ighm	immunoglobulin heavy constant mu	16019	88	124	211	108	189	14621	135.31	< 0.0001

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT Aorta 6 weeks	Mean WT Aorta 32 weeks	Mean WT Aorta 78 weeks	Mean ApoE$^{-/-}$ Aorta 6 weeks	Mean ApoE$^{-/-}$ Aorta 32 weeks	Mean ApoE$^{-/-}$ Aorta 78 weeks	Fold change ApoE$^{-/-}$ Aorta 78 weeks vs. 6 weeks	p ANOVA
1425324_x_at	Ighm	immunoglobulin heavy constant mu	16019	49	77	38	34	66	4275	126.80	0.0007
1427756_x_at	Ighm	immunoglobulin heavy constant mu	16019	82	67	73	51	91	4981	97.95	< 0.0001
1448576_at	Il7r	interleukin 7 receptor	16197	9	10	17	21	471	938	45.04	< 0.0001
1427329_a_at	Ighm	immunoglobulin heavy constant mu	16019	543	718	1806	648	992	17344	26.75	< 0.0001
1427351_s_at	Ighm	immunoglobulin heavy constant mu	16019	444	654	3608	658	1452	15930	24.20	0.001
1448575_at	Il7r	interleukin 7 receptor	16197	106	150	203	136	1187	3130	22.99	< 0.0001
1423182_at	Tnfrsf13b	tumor necrosis factor receptor superfamily, member 13b	57916	39	53	123	46	162	647	14.17	< 0.0001
1427870_x_at	Ighm	immunoglobulin heavy constant mu	16019	600	957	1072	623	660	7671	12.32	< 0.0001
1428787_at	Nckap1l	NCK associated protein 1 like	105855	130	86	249	159	779	1589	10.00	< 0.0001
1418830_at	Cd79a	CD79A antigen (immunoglobulin-associated alpha)	12518	112	143	394	127	150	1235	9.73	0.0001
1422124_a_at	Ptprc	protein tyrosine phosphatase, receptor type, C	19264	486	772	990	642	2454	5106	7.95	< 0.0001
1422631_at	Ahr	aryl-hydrocarbon receptor	11622	227	284	231	185	675	1161	6.29	< 0.0001

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT Aorta 6 weeks	Mean WT Aorta 32 weeks	Mean WT Aorta 78 weeks	Mean ApoE$^{-/-}$ Aorta 6 weeks	Mean ApoE$^{-/-}$ Aorta 32 weeks	Mean ApoE$^{-/-}$ Aorta 78 weeks	Fold change ApoE$^{-/-}$ Aorta 78 weeks vs. 6 weeks	p ANOVA
1425519_a_at	Cd74	CD74 antigen (invariant polypeptide of major histocompatibility complex, class II antigen-associated)	16149	1634	2699	4537	1955	7675	11699	5.98	< 0.0001
1428786_at	Nckap1l	NCK associated protein 1 like	105855	283	375	583	364	896	2157	5.92	< 0.0001
1435477_s_at	Fcgr2b	Fc receptor, IgG, low affinity IIb	14130	736	720	1048	705	2176	3922	5.56	< 0.0001
1436861_at	Il7	interleukin 7	16196	73	104	123	46	135	240	5.23	0.0001
1451941_a_at	Fcgr2b	Fc receptor, IgG, low affinity IIb	14130	549	458	690	506	1472	2594	5.13	< 0.0001
1455332_x_at	Fcgr2b	Fc receptor, IgG, low affinity IIb	14130	254	277	421	334	907	1444	4.32	< 0.0001
1427007_at	Sash3	SAM and SH3 domain containing 3	74131	288	345	516	338	740	1354	4.00	< 0.0001
1418110_a_at	Inpp5d	inositol polyphosphate-5-phosphatase D	16331	345	428	498	344	752	1281	3.72	< 0.0001
1450136_at	Cd38	CD38 antigen	12494	141	123	131	177	578	644	3.64	< 0.0001
1433741_at	Cd38	CD38 antigen	12494	414	530	662	531	1318	1894	3.57	< 0.0001
1455656_at	Btla	B and T lymphocyte associated	208154	218	287	373	282	342	1006	3.57	0.0002
1449473_s_at	Cd40	CD40 antigen	21939	84	45	100	68	145	230	3.39	0.03

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT Aorta 6 weeks	Mean WT Aorta 32 weeks	Mean WT Aorta 78 weeks	Mean ApoE$^{-/-}$ Aorta 6 weeks	Mean ApoE$^{-/-}$ Aorta 32 weeks	Mean ApoE$^{-/-}$ Aorta 78 weeks	Fold change ApoE$^{-/-}$ Aorta 78 weeks vs. 6 weeks	p ANOVA
1420425_at	Prdm1	PR domain containing 1, with ZNF domain	12142	144	169	175	159	193	463	2.92	0.002
1452535_at	Ighm	immunoglobulin heavy constant mu	16019	171	163	236	175	123	494	2.82	0.001
1419307_at	Tnfrsf13c	tumor necrosis factor receptor superfamily, member 13c	72049	141	192	284	171	183	472	2.76	0.005
1460255_at	Tnfsf13b	tumor necrosis factor (ligand) superfamily, member 13b	24099	340	449	496	381	613	967	2.54	< 0.0001
1417122_at	Vav3	vav 3 oncogene	57257	150	247	205	178	308	447	2.51	0.001
1427164_at	Il13ra1	interleukin 13 receptor, alpha 1	16164	234	144	152	186	325	463	2.49	< 0.0001
1439205_at	Nfatc2	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 2	18019	218	264	301	221	364	538	2.43	0.0006
1448600_s_at	Vav3	vav 3 oncogene	57257	212	366	305	250	365	542	2.17	0.0006
1460415_a_at	Cd40	CD40 antigen	21939	95	54	83	119	130	222	1.88	0.01
1454783_at	Il13ra1	interleukin 13 receptor, alpha 1	16164	674	636	504	582	1076	1069	1.84	< 0.0001
1424195_a_at	Inpp5d	inositol polyphosphate-5-phosphatase D	16331	277	295	192	234	344	410	1.75	0.0001

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT Aorta 6 weeks	Mean WT Aorta 32 weeks	Mean WT Aorta 78 weeks	Mean ApoE$^{-/-}$ Aorta 6 weeks	Mean ApoE$^{-/-}$ Aorta 32 weeks	Mean ApoE$^{-/-}$ Aorta 78 weeks	Fold change ApoE$^{-/-}$ Aorta 78 weeks vs. 6 weeks	p ANOVA
1437270_a_at	Clcf1	cardiotrophin-like cytokine factor 1	56708	122	162	123	194	244	248	1.28	0.001
1421818_at	Bcl6	B cell leukemia/lymphoma 6	12053	1904	700	919	1243	862	1208	-1.03	0.007
1431061_s_at	Peli1	pellino 1	67245	503	198	269	387	222	270	-1.43	0.002
1450381_a_at	Bcl6	B cell leukemia/lymphoma 6	12053	843	384	390	624	366	434	-1.44	0.0005
1442827_at	Tlr4	toll-like receptor 4	21898	413	183	137	320	208	142	-2.26	0.0009
1421028_a_at	Mef2c	myocyte enhancer factor 2C	17260	1504	712	697	1362	767	561	-2.43	< 0.0001
1421027_a_at	Mef2c	myocyte enhancer factor 2C	17260	2673	1597	972	2514	1694	854	-2.94	< 0.0001

F) B cell differentiation

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT Aorta 6 weeks	Mean WT Aorta 32 weeks	Mean WT Aorta 78 weeks	Mean ApoE$^{-/-}$ Aorta 6 weeks	Mean ApoE$^{-/-}$ Aorta 32 weeks	Mean ApoE$^{-/-}$ Aorta 78 weeks	Fold change ApoE$^{-/-}$ Aorta 78 weeks vs. 6 weeks	p ANOVA
1460188_at	Ptpn6	protein tyrosine phosphatase, non-	15170	37	51	198	46	567	1324	28.96	< 0.0001

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT Aorta 6 weeks	Mean WT Aorta 32 weeks	Mean WT Aorta 78 weeks	Mean ApoE$^{-/-}$ Aorta 6 weeks	Mean ApoE$^{-/-}$ Aorta 32 weeks	Mean ApoE$^{-/-}$ Aorta 78 weeks	Fold change ApoE$^{-/-}$ Aorta 78 weeks vs. 6 weeks	p ANOVA
		receptor type 6									
1436312_at	Ikzf1	IKAROS family zinc finger 1	22778	40	43	212	35	338	948	27.12	< 0.0001
1425477_x_at	H2-Ab1	histocompatibility 2, class II antigen A, beta 1	14961	279	361	491	263	1416	3555	13.53	< 0.0001
1428787_at	Nckap1l	NCK associated protein 1 like	105855	130	86	249	159	779	1589	10.00	< 0.0001
1451721_a_at	H2-Ab1	histocompatibility 2, class II antigen A, beta 1	14961	457	616	941	514	2144	5102	9.93	< 0.0001
1418830_at	Cd79a	CD79A antigen (immunoglobulin-associated alpha)	12518	112	143	394	127	150	1235	9.73	0.0001
1450648_s_at	H2-Ab1	histocompatibility 2, class II antigen A, beta 1	14961	780	1005	1459	782	2234	6399	8.19	< 0.0001
1422124_a_at	Ptprc	protein tyrosine phosphatase, receptor type, C	19264	486	772	990	642	2454	5106	7.95	< 0.0001
1416296_at	Il2rg	interleukin 2 receptor, gamma chain	16186	281	418	374	235	769	1613	6.86	0.003
1418261_at	Sykb	spleen tyrosine kinase	20963	311	375	517	281	875	1860	6.63	< 0.0001
1428786_at	Nckap1l	NCK associated protein 1 like	105855	283	375	583	364	896	2157	5.92	< 0.0001

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT Aorta 6 weeks	Mean WT Aorta 32 weeks	Mean WT Aorta 78 weeks	Mean ApoE$^{-/-}$ Aorta 6 weeks	Mean ApoE$^{-/-}$ Aorta 32 weeks	Mean ApoE$^{-/-}$ Aorta 78 weeks	Fold change ApoE$^{-/-}$ Aorta 78 weeks vs. 6 weeks	p ANOVA
1437356_at	Gpr183	G protein-coupled receptor 183	321019	103	133	222	107	250	617	5.75	< 0.0001
1418133_at	Bcl3	B cell leukemia/lymphoma 3	12051	42	138	136	80	335	461	5.73	0.0006
1416295_a_at	Il2rg	interleukin 2 receptor, gamma chain	16186	369	525	720	475	1479	2625	5.53	< 0.0001
1418262_at	Sykb	spleen tyrosine kinase	20963	149	133	185	133	404	664	4.98	< 0.0001
1418110_a_at	Inpp5d	inositol polyphosphate-5-phosphatase D	16331	345	428	498	344	752	1281	3.72	< 0.0001
1456694_x_at	Ptpn6	protein tyrosine phosphatase, non-receptor type 6	15170	449	549	568	367	660	1075	2.93	0.001
1420425_at	Prdm1	PR domain containing 1, with ZNF domain	12142	144	169	175	159	193	463	2.92	0.002
1423319_at	Hhex	hematopoietically expressed homeobox	15242	379	409	524	346	644	902	2.61	0.0001
1433699_at	Tnfaip3	tumor necrosis factor, alpha-induced protein 3	21929	569	683	845	643	1032	1441	2.24	0.0001
1450829_at	Tnfaip3	tumor necrosis factor, alpha-induced protein 3	21929	50	77	119	133	144	239	1.80	0.01
1424195_a_at	Inpp5d	inositol polyphosphate-5-	16331	277	295	192	234	344	410	1.75	0.0001

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT Aorta 6 weeks	Mean WT Aorta 32 weeks	Mean WT Aorta 78 weeks	Mean ApoE$^{-/-}$ Aorta 6 weeks	Mean ApoE$^{-/-}$ Aorta 32 weeks	Mean ApoE$^{-/-}$ Aorta 78 weeks	Fold change ApoE$^{-/-}$ Aorta 78 weeks vs. 6 weeks	p ANOVA
		phosphatase D									
1437270_a_at	Clcf1	cardiotrophin-like cytokine factor 1	56708	122	162	123	194	244	248	1.28	0.001
1421818_at	Bcl6	B cell leukemia/lymphoma 6	12053	1904	700	919	1243	862	1208	-1.03	0.007
1448395_at	Sfrp1	secreted frizzled-related protein 1	20377	1584	999	626	1162	1758	870	-1.33	0.0003
1421857_at	Adam17	A disintegrin and metallopeptidase domain 17	11491	849	443	318	746	452	556	-1.34	0.0003
1456429_at	Malt1	mucosa associated lymphoid tissue lymphoma translocation gene 1	240354	466	236	169	385	312	278	-1.39	0.02
1450381_a_at	Bcl6	B cell leukemia/lymphoma 6	12053	843	384	390	624	366	434	-1.44	0.0005
1416594_at	Sfrp1	secreted frizzled-related protein 1	20377	476	46	81	379	384	230	-1.64	< 0.0001
1422102_a_at	Stat5b	signal transducer and activator of transcription 5B	20851	326	292	265	404	190	213	-1.89	0.002
1434572_at	Hdac9	histone deacetylase 9	79221	1063	818	854	827	532	419	-1.98	0.0001
1421306_a_at	Hdac9	histone deacetylase 9	79221	305	172	99	215	135	54	-3.94	0.0005

A2: Aging-spleen: Differential expression of transcripts coding B cell regulating genes in WT and ApoE$^{-/-}$ spleen (Fig.S1A,B).

A) B cell mediated immunity

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT spleen 32 weeks	Mean WT spleen 78 weeks	Mean ApoE$^{-/-}$ spleen 32 weeks	Mean ApoE$^{-/-}$ spleen 78 weeks	Fold change ApoE$^{-/-}$ spleen 78 weeks vs. 32 weeks	p ANOVA
1440165_at	Ptprc	protein tyrosine phosphatase, receptor type, C	19264	218	80	161	86	-1.88	0.02
1451713_a_at	Fcer2a	Fc receptor, IgE, low affinity II, alpha polypeptide	14128	1410	900	793	423	-1.88	0.002
1420653_at	Tgfb1	transforming groWTh factor, beta 1	21803	3058	1497	3033	1566	-1.94	0.0003
1420577_at	Aicda	activation-induced cytidine deaminase	11628	307	192	459	160	-2.87	0.002

B) B cell activation

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT spleen 32 weeks	Mean WT spleen 78 weeks	Mean ApoE$^{-/-}$ spleen 32 weeks	Mean ApoE$^{-/-}$ spleen 78 weeks	Fold change ApoE$^{-/-}$ spleen 78 weeks vs.	p ANOVA

								32 weeks	
1460187_at	Sfrp1	secreted frizzled-related protein 1	20377	486	672	404	963	2.38	0.01
1426910_at	Pawr	PRKC, apoptosis, WT1, regulator	114774	353	681	366	841	2.30	0.0002
1417122_at	Vav3	vav 3 oncogene	57257	204	429	211	472	2.24	0.001
1427164_at	Il13ra1	interleukin 13 receptor, alpha 1	16164	90	201	144	303	2.10	0.008
1451737_at	Pik3r1	phosphatidylinositol 3-kinase, regulatory subunit, polypeptide 1 (p85 alpha)	18708	687	313	494	295	-1.68	0.005
1456429_at	Malt1	mucosa associated lymphoid tissue lymphoma translocation gene 1	240354	2161	986	1831	992	-1.85	0.004
1440165_at	Ptprc	protein tyrosine phosphatase, receptor type, C	19264	218	80	161	86	-1.88	0.02
1420653_at	Tgfb1	transforming groWTh factor, beta 1	21803	3058	1497	3033	1566	-1.94	0.0003
1445068_at	Malt1	mucosa associated lymphoid tissue lymphoma translocation gene 1	240354	1491	612	1058	532	-1.99	0.005
1425797_a_at	Sykb	spleen tyrosine kinase	20963	1587	610	1306	598	-2.18	0.001
1427764_a_at	Tcf3	transcription factor 3	21423	882	317	655	286	-2.29	0.0002
1420577_at	Aicda	activation-induced cytidine deaminase	11628	307	192	459	160	-2.87	0.002

C) Positive regulation of B cell mediated immunity

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT spleen 32 weeks	Mean WT spleen 78 weeks	Mean ApoE$^{-/-}$ spleen 32 weeks	Mean ApoE$^{-/-}$ spleen 78 weeks	Fold change ApoE$^{-/-}$ spleen 78 weeks vs. 32 weeks	p ANOVA
1440165_at	Ptprc	protein tyrosine phosphatase, receptor type, C	19264	218	80	161	86	-1.88	0.02
1451713_a_at	Fcer2a	Fc receptor, IgE, low affinity II, alpha polypeptide	14128	1410	900	793	423	-1.88	0.002

D) Positive regulation of B cell activation

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT spleen 32 weeks	Mean WT spleen 78 weeks	Mean ApoE$^{-/-}$ spleen 32 weeks	Mean ApoE$^{-/-}$ spleen 78 weeks	Fold change ApoE$^{-/-}$ spleen 78 weeks vs. 32 weeks	p ANOVA
1417122_at	Vav3	vav 3 oncogene	57257	204	429	211	472	2.24	0.001
1427164_at	Il13ra1	interleukin 13 receptor, alpha 1	16164	90	201	144	303	2.10	0.008
1440165_at	Ptprc	protein tyrosine phosphatase, receptor	19264	218	80	161	86	-1.88	0.02

type, C

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT spleen 32 weeks	Mean WT spleen 78 weeks	Mean ApoE$^{-/-}$ spleen 32 weeks	Mean ApoE$^{-/-}$ spleen 78 weeks	Fold change ApoE$^{-/-}$ spleen 78 weeks vs. 32 weeks	p ANOVA
1420653_at	Tgfb1	transforming groWTh factor, beta 1	21803	3058	1497	3033	1566	-1.94	0.0003
1425797_a_at	Sykb	spleen tyrosine kinase	20963	1587	610	1306	598	-2.18	0.001
1427764_a_at	Tcf3	transcription factor 3	21423	882	317	655	286	-2.29	0.0002

E) B cell proliferation

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT spleen 32 weeks	Mean WT spleen 78 weeks	Mean ApoE$^{-/-}$ spleen 32 weeks	Mean ApoE$^{-/-}$ spleen 78 weeks	Fold change ApoE$^{-/-}$ spleen 78 weeks vs. 32 weeks	p ANOVA
1426910_at	Pawr	PRKC, apoptosis, WT1, regulator	114774	353	681	366	841	2.30	0.0002
1417122_at	Vav3	vav 3 oncogene	57257	204	429	211	472	2.24	0.001
1427164_at	Il13ra1	interleukin 13 receptor, alpha 1	16164	90	201	144	303	2.10	0.008
1440165_at	Ptprc	protein tyrosine phosphatase, receptor type, C	19264	218	80	161	86	-1.88	0.02
1427764_a_at	Tcf3	transcription factor 3	21423	882	317	655	286	-2.29	0.0002

F) B cell differentiation

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT spleen 32 weeks	Mean WT spleen 78 weeks	Mean ApoE$^{-/-}$ spleen 32 weeks	Mean ApoE$^{-/-}$ spleen 78 weeks	Fold change ApoE$^{-/-}$ spleen 78 weeks vs. 32 weeks	p ANOVA
1460187_at	Sfrp1	secreted frizzled-related protein 1	20377	486	672	404	963	2.38	0.01
1451737_at	Pik3r1	phosphatidylinositol 3-kinase, regulatory subunit, polypeptide 1 (p85 alpha)	18708	687	313	494	295	-1.68	0.005
1456429_at	Malt1	mucosa associated lymphoid tissue lymphoma translocation gene 1	240354	2161	986	1831	992	-1.85	0.004
1440165_at	Ptprc	protein tyrosine phosphatase, receptor type, C	19264	218	80	161	86	-1.88	0.02
1445068_at	Malt1	mucosa associated lymphoid tissue lymphoma translocation gene 1	240354	1491	612	1058	532	-1.99	0.005
1425797_a_at	Sykb	spleen tyrosine kinase	20963	1587	610	1306	598	-2.18	0.001
1427764_a_at	Tcf3	transcription factor 3	21423	882	317	655	286	-2.29	0.0002

A3: Aging-blood: Differential expression of transcripts coding B cell regulating genes in WT and ApoE$^{-/-}$ blood (Fig.S1C,D).

A) B cell mediated immunity

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT blood 32 weeks	Mean WT blood 78 weeks	Mean ApoE$^{-/-}$ blood 32 weeks	Mean ApoE$^{-/-}$ blood 78 weeks	Fold change ApoE$^{-/-}$ blood 78 weeks vs. 32 weeks	p ANOVA
1417876_at	Fcgr1	Fc receptor, IgG, high affinity I	14129	412	423	718	915	1.27	0.01
1417244_a_at	Irf7	interferon regulatory factor 7	54123	1415	1063	2119	2151	1.02	0.01
1440165_at	Ptprc	protein tyrosine phosphatase, receptor type, C	19264	710	61	589	171	-3.44	0.02

B) B cell activation

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT blood 32 weeks	Mean WT blood 78 weeks	Mean ApoE$^{-/-}$ blood 32 weeks	Mean ApoE$^{-/-}$ blood 78 weeks	Fold change ApoE$^{-/-}$ blood 78 weeks vs. 32 weeks	p ANOVA
1425714_a_at	Nfam1	Nfat activating molecule with ITAM motif 1	74039	1118	355	1011	1007	-1.00	0.03

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT blood 32 weeks	Mean WT blood 78 weeks	Mean ApoE$^{-/-}$ blood 32 weeks	Mean ApoE$^{-/-}$ blood 78 weeks	Fold change	p ANOVA
1421140_a_at	Foxp1	forkhead box P1	108655	2904	1277	2116	1360	-1.56	0.04
1438802_at	Foxp1	forkhead box P1	108655	1653	740	1582	646	-2.45	0.006
1445068_at	Malt1	mucosa associated lymphoid tissue lymphoma translocation gene 1	240354	1845	409	1463	558	-2.62	0.01
1457239_at	Sykb	spleen tyrosine kinase	20963	183	48	292	86	-3.38	0.04
1440165_at	Ptprc	protein tyrosine phosphatase, receptor type, C	19264	710	61	589	171	-3.44	0.02

C) Positive regulation of B cell mediated immunity

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT blood 32 weeks	Mean WT blood 78 weeks	Mean ApoE$^{-/-}$ blood 32 weeks	Mean ApoE$^{-/-}$ blood 78 weeks	Fold change ApoE$^{-/-}$ blood 78 weeks vs. 32 weeks	p ANOVA
1417876_at	Fcgr1	Fc receptor, IgG, high affinity I	14129	412	423	718	915	1.27	0.01
1440165_at	Ptprc	protein tyrosine phosphatase, receptor type, C	19264	710	61	589	171	-3.44	0.02

D) Positive regulation of B cell activation

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT blood 32 weeks	Mean WT blood 78 weeks	Mean ApoE$^{-/-}$ blood 32 weeks	Mean ApoE$^{-/-}$ blood 78 weeks	Fold change ApoE$^{-/-}$ blood 78 weeks vs. 32 weeks	p ANOVA
1457239_at	Sykb	spleen tyrosine kinase	20963	183	48	292	86	-3.38	0.04
1440165_at	Ptprc	protein tyrosine phosphatase, receptor type, C	19264	710	61	589	171	-3.44	0.02

E) B cell differentiation

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT blood 32 weeks	Mean WT blood 78 weeks	Mean ApoE$^{-/-}$ blood 32 weeks	Mean ApoE$^{-/-}$ blood 78 weeks	Fold change ApoE$^{-/-}$ blood 78 weeks vs. 32 weeks	p ANOVA
1425714_a_at	Nfam1	Nfat activating molecule with ITAM motif 1	74039	1118	355	1011	1007	-1.00	0.03
1421140_a_at	Foxp1	forkhead box P1	108655	2904	1277	2116	1360	-1.56	0.04
1438802_at	Foxp1	forkhead box P1	108655	1653	740	1582	646	-2.45	0.006
1445068_at	Malt1	mucosa associated lymphoid tissue	240354	1845	409	1463	558	-2.62	0.01

lymphoma translocation gene 1

1457239_at	Sykb	spleen tyrosine kinase	20963	183	48	292	86	-3.38	0.04
1440165_at	Ptprc	protein tyrosine phosphatase, receptor type, C	19264	710	61	589	171	-3.44	0.02

B. Transcript atlas of WT adventitia, ApoE$^{-/-}$ adventitia without plaque, plaque, and ATLOs reveal the territoriality of B cell immunity in the diseased aorta (Fig.2).

B1: Adventitia cluster: Differential expression of transcripts coding for B cell-regulating genes in WT and ApoE$^{-/-}$ aorta adventitia:

A) B cell mediated immunity

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT adventitia	Mean ApoE$^{-/-}$ adventitia no plaque	Mean ApoE$^{-/-}$ ATLO	Fold change ApoE$^{-/-}$ ATLO vs. ApoE$^{-/-}$ adventitia no plaque	p ANOVA
1425324_x_at	Ighm	immunoglobulin heavy constant mu	16019	317	284	7656	26.93	0.04
1427869_at	Ighm	immunoglobulin heavy constant mu	16019	11	12	246	20.65	0.01
1427870_x_at	Ighm	immunoglobulin heavy constant mu	16019	1315	816	10203	12.50	0.05
1425477_x_at	H2-Ab1	histocompatibility 2, class II antigen A, beta 1	14961	235	269	2613	9.70	0.0004

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT adventitia	Mean ApoE$^{-/-}$ adventitia no plaque	Mean ApoE$^{-/-}$ ATLO	Fold change ApoE$^{-/-}$ ATLO vs. ApoE$^{-/-}$ adventitia no plaque	p ANOVA
1460188_at	Ptpn6	protein tyrosine phosphatase, non-receptor type 6	15170	150	105	926	8.78	0.004
1424302_at	Lilrb3	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 3	18733	102	184	1245	6.77	< 0.0001
1422124_a_at	Ptprc	protein tyrosine phosphatase, receptor type, C	19264	941	1071	6773	6.32	0.0004
1451721_a_at	H2-Ab1	histocompatibility 2, class II antigen A, beta 1	14961	435	582	3528	6.06	0.0008
1455332_x_at	Fcgr2b	Fc receptor, IgG, low affinity IIb	14130	252	390	2245	5.75	< 0.0001
1451941_a_at	Fcgr2b	Fc receptor, IgG, low affinity IIb	14130	282	355	1782	5.01	0.0002
1435477_s_at	Fcgr2b	Fc receptor, IgG, low affinity IIb	14130	695	1072	5035	4.70	< 0.0001
1422527_at	H2-DMa	histocompatibility 2, class II, locus DMa	14998	297	361	1653	4.57	0.0002
1417597_at	Cd28	CD28 antigen	12487	72	98	446	4.55	0.002
1437270_a_at	Clcf1	cardiotrophin-like cytokine factor 1	56708	59	64	284	4.42	0.002
1417876_at	Fcgr1	Fc receptor, IgG, high affinity I	14129	120	176	735	4.18	0.0004
1425519_a_at	Cd74	CD74 antigen (invariant polypeptide of major histocompatibility complex, class II antigen-associated)	16149	3626	4458	18504	4.15	0.0002

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT adventitia	Mean ApoE$^{-/-}$ adventitia no plaque	Mean ApoE$^{-/-}$ ATLO	Fold change ApoE$^{-/-}$ ATLO vs. ApoE$^{-/-}$ adventitia no plaque	p ANOVA
1423543_at	Swap70	SWA-70 protein	20947	199	158	655	4.14	0.002
1450648_s_at	H2-Ab1	histocompatibility 2, class II antigen A, beta 1	14961	1634	1981	7773	3.92	0.0002
1435476_a_at	Fcgr2b	Fc receptor, IgG, low affinity IIb	14130	829	971	3572	3.68	< 0.0001
1449473_s_at	Cd40	CD40 antigen	21939	86	105	376	3.58	0.0003
1418133_at	Bcl3	B cell leukemia/lymphoma 3	12051	96	165	570	3.45	0.001
1434366_x_at	C1qb	complement component 1, q subcomponent, beta polypeptide	12260	1731	2698	8974	3.33	0.0003
1417063_at	C1qb	complement component 1, q subcomponent, beta polypeptide	12260	903	1570	5054	3.22	0.0002
1418340_at	Fcer1g	Fc receptor, IgE, high affinity I, gamma polypeptide	14127	435	682	2149	3.15	0.0004
1427329_a_at	Ighm	immunoglobulin heavy constant mu	16019	8628	9893	30659	3.10	0.03
1448620_at	Fcgr3	Fc receptor, IgG, low affinity III	14131	431	693	1955	2.82	0.001
1417381_at	C1qa	complement component 1, q subcomponent, alpha polypeptide	12259	1022	1724	4626	2.68	0.002
1437726_x_at	C1qb	complement component 1, q subcomponent, beta polypeptide	12260	2201	3878	10107	2.61	0.0003

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT adventitia	Mean ApoE$^{-/-}$ adventitia no plaque	Mean ApoE$^{-/-}$ ATLO	Fold change ApoE$^{-/-}$ ATLO vs. ApoE$^{-/-}$ adventitia no plaque	p ANOVA
1418021_at	C4b	complement component 4B (Childo blood group)	12268	3136	4259	10968	2.58	< 0.0001
1417009_at	C1ra	complement component 1, r subcomponent A	50909	569	804	2035	2.53	0.0003
1423954_at	C3	complement component 3	12266	5736	7932	18139	2.29	< 0.0001
1422847_a_at	Prkcd	protein kinase C, delta	18753	496	489	1036	2.12	0.0005
1424041_s_at	C1s	complement component 1, s subcomponent	50908	1333	1738	3663	2.11	0.0009
1419272_at	Myd88	myeloid differentiation primary response gene 88	17874	711	761	1601	2.10	0.0002
1416625_at	Serping1	serine (or cysteine) peptidase inhibitor, clade G, member 1	12258	5258	7199	13937	1.94	0.006
1416051_at	C2	complement component 2 (within H-2S)	12263	765	858	1652	1.92	0.009
1456694_x_at	Ptpn6	protein tyrosine phosphatase, non-receptor type 6	15170	414	568	1084	1.91	0.004
1418110_a_at	Inpp5d	inositol polyphosphate-5-phosphatase D	16331	362	433	821	1.90	0.002
1419212_at	Icosl	icos ligand	50723	271	376	592	1.57	0.004
1421775_at	Fcer1a	Fc receptor, IgE, high affinity I, alpha polypeptide	14125	52	202	134	-1.51	0.02
1424456_at	Pvrl2	poliovirus receptor-related 2	19294	177	287	138	-2.07	0.02

B) B cell activation

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT adventitia	Mean ApoE$^{-/-}$ adventitia no plaque	Mean ApoE$^{-/-}$ ATLO	Fold change ApoE$^{-/-}$ ATLO vs. ApoE$^{-/-}$ adventitia no plaque	p ANOVA
1425324_x_at	Ighm	immunoglobulin heavy constant mu	16019	317	284	7656	26.93	0.04
1427869_at	Ighm	immunoglobulin heavy constant mu	16019	11	12	246	20.65	0.01
1427870_x_at	Ighm	immunoglobulin heavy constant mu	16019	1315	816	10203	12.50	0.05
1448576_at	Il7r	interleukin 7 receptor	16197	11	24	270	11.10	0.02
1425477_x_at	H2-Ab1	histocompatibility 2, class II antigen A, beta 1	14961	235	269	2613	9.70	0.0004
1448575_at	Il7r	interleukin 7 receptor	16197	140	168	1546	9.22	0.0002
1460188_at	Ptpn6	protein tyrosine phosphatase, non-receptor type 6	15170	150	105	926	8.78	0.004
1418830_at	Cd79a	CD79A antigen (immunoglobulin-associated alpha)	12518	229	158	1276	8.09	0.003
1423182_at	Tnfrsf13b	tumor necrosis factor receptor superfamily, member 13b	57916	143	130	959	7.38	0.002
1419406_a_at	Bcl11a	B cell CLL/lymphoma 11A (zinc finger protein)	14025	94	42	306	7.25	0.03
1423226_at	Ms4a1	membrane-spanning 4-domains, subfamily A, member 1	12482	406	260	1842	7.08	0.04

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT adventitia	Mean ApoE$^{-/-}$ adventitia no plaque	Mean ApoE$^{-/-}$ ATLO	Fold change ApoE$^{-/-}$ ATLO vs. ApoE$^{-/-}$ adventitia no plaque	p ANOVA
1422124_a_at	Ptprc	protein tyrosine phosphatase, receptor type, C	19264	941	1071	6773	6.32	0.0004
1451721_a_at	H2-Ab1	histocompatibility 2, class II antigen A, beta 1	14961	435	582	3528	6.06	0.0008
1416034_at	Cd24a	CD24a antigen	12484	721	222	1324	5.95	0.02
1455332_x_at	Fcgr2b	Fc receptor, IgG, low affinity IIb	14130	252	390	2245	5.75	< 0.0001
1417976_at	Ada	adenosine deaminase	11486	68	66	354	5.36	0.008
1419307_at	Tnfrsf13c	tumor necrosis factor receptor superfamily, member 13c	72049	215	186	950	5.10	0.01
1451780_at	Blnk	B cell linker	17060	363	356	1796	5.04	0.001
1451941_a_at	Fcgr2b	Fc receptor, IgG, low affinity IIb	14130	282	355	1782	5.01	0.0002
1435477_s_at	Fcgr2b	Fc receptor, IgG, low affinity IIb	14130	695	1072	5035	4.70	< 0.0001
1417597_at	Cd28	CD28 antigen	12487	72	98	446	4.55	0.002
1419538_at	Flt3	FMS-like tyrosine kinase 3	14255	71	52	237	4.52	0.02
1416295_a_at	Il2rg	interleukin 2 receptor, gamma chain	16186	665	756	3396	4.49	0.001
1437270_a_at	Clcf1	cardiotrophin-like cytokine factor 1	56708	59	64	284	4.42	0.002

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT adventitia	Mean ApoE$^{-/-}$ adventitia no plaque	Mean ApoE$^{-/-}$ ATLO	Fold change ApoE$^{-/-}$ ATLO vs. ApoE$^{-/-}$ adventitia no plaque	p ANOVA
1427164_at	Il13ra1	interleukin 13 receptor, alpha 1	16164	52	61	268	4.40	0.0009
1460255_at	Tnfsf13b	tumor necrosis factor (ligand) superfamily, member 13b	24099	177	239	1045	4.38	0.0001
1448182_a_at	Cd24a	CD24a antigen	12484	2648	898	3859	4.30	0.004
1428787_at	Nckap1l	NCK associated protein 1 like	105855	151	163	702	4.30	0.0007
1416296_at	Il2rg	interleukin 2 receptor, gamma chain	16186	401	329	1410	4.28	0.003
1425519_a_at	Cd74	CD74 antigen (invariant polypeptide of major histocompatibility complex, class II antigen-associated)	16149	3626	4458	18504	4.15	0.0002
1423543_at	Swap70	SWA-70 protein	20947	199	158	655	4.14	0.002
1418261_at	Sykb	spleen tyrosine kinase	20963	307	392	1601	4.08	0.0002
1437502_x_at	Cd24a	CD24a antigen	12484	1303	540	2187	4.05	0.002
1450648_s_at	H2-Ab1	histocompatibility 2, class II antigen A, beta 1	14961	1634	1981	7773	3.92	0.0002
1435476_a_at	Fcgr2b	Fc receptor, IgG, low affinity IIb	14130	829	971	3572	3.68	< 0.0001
1420425_at	Prdm1	PR domain containing 1, with ZNF domain	12142	126	110	402	3.66	0.002

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT adventitia	Mean ApoE$^{-/-}$ adventitia no plaque	Mean ApoE$^{-/-}$ ATLO	Fold change ApoE$^{-/-}$ ATLO vs. ApoE$^{-/-}$ adventitia no plaque	p ANOVA
1449473_s_at	Cd40	CD40 antigen	21939	86	105	376	3.58	0.0003
1418133_at	Bcl3	B cell leukemia/lymphoma 3	12051	96	165	570	3.45	0.001
1418262_at	Sykb	spleen tyrosine kinase	20963	108	118	401	3.39	0.002
1427007_at	Sash3	SAM and SH3 domain containing 3	74131	394	423	1420	3.36	0.0004
1428786_at	Nckap1l	NCK associated protein 1 like	105855	289	404	1335	3.31	< 0.0001
1454783_at	Il13ra1	interleukin 13 receptor, alpha 1	16164	116	144	457	3.17	0.0008
1427329_a_at	Ighm	immunoglobulin heavy constant mu	16019	8628	9893	30659	3.10	0.03
1422003_at	Cxcr5	chemokine (C-X-C motif) receptor 5	12145	77	95	294	3.08	0.02
1420351_at	Tnfrsf4	tumor necrosis factor receptor superfamily, member 4	22163	292	232	710	3.06	0.002
1453281_at	Pik3cd	phosphatidylinositol 3-kinase catalytic delta polypeptide	18707	161	186	565	3.04	< 0.0001
1460419_a_at	Prkcb	protein kinase C, beta	18751	603	528	1599	3.03	0.0003
1426926_at	Plcg2	phospholipase C, gamma 2	234779	494	418	1220	2.92	0.0002
1433741_at	Cd38	CD38 antigen	12494	499	589	1580	2.68	0.0002

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT adventitia	Mean ApoE$^{-/-}$ adventitia no plaque	Mean ApoE$^{-/-}$ ATLO	Fold change ApoE$^{-/-}$ ATLO vs. ApoE$^{-/-}$ adventitia no plaque	p ANOVA
1420404_at	Cd86	CD86 antigen	12524	129	167	422	2.53	0.0002
1423478_at	Prkcb	protein kinase C, beta	18751	139	171	414	2.42	0.007
1421141_a_at	Foxp1	forkhead box P1	108655	93	86	197	2.30	0.02
1421858_at	Adam17	a disintegrin and metallopeptidase domain 17	11491	429	477	1039	2.18	0.0003
1421205_at	Atm	ataxia telangiectasia mutated homolog (human)	11920	218	150	319	2.13	0.03
1422847_a_at	Prkcd	protein kinase C, delta	18753	496	489	1036	2.12	0.0005
1456694_x_at	Ptpn6	protein tyrosine phosphatase, non-receptor type 6	15170	414	568	1084	1.91	0.004
1418110_a_at	Inpp5d	inositol polyphosphate-5-phosphatase D	16331	362	433	821	1.90	0.002
1435176_a_at	Id2	inhibitor of DNA binding 2	15902	1205	1571	2573	1.64	0.006
1419212_at	Icosl	icos ligand	50723	271	376	592	1.57	0.004
1421999_at	Tshr	thyroid stimulating hormone receptor	22095	213	278	122	-2.28	0.005

C) Immunoglobulin

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT adventitia	Mean ApoE$^{-/-}$ adventitia no plaque	Mean ApoE$^{-/-}$ ATLO	Fold change ApoE$^{-/-}$ ATLO vs. ApoE$^{-/-}$ adventitia no plaque	p ANOVA
1425324_x_at	Ighm	immunoglobulin heavy constant mu	16019	317	284	7656	26.93	0.04
1427869_at	Ighm	immunoglobulin heavy constant mu	16019	11	12	246	20.65	0.01
1424305_at	Igj	immunoglobulin joining chain	16069	6602	4731	21748	4.60	0.002
1427329_a_at	Ighm	immunoglobulin heavy constant mu	16019	8628	9893	30659	3.10	0.03

D) Positive regulation of B cell mediated immunity

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT adventitia	Mean ApoE$^{-/-}$ adventitia no plaque	Mean ApoE$^{-/-}$ ATLO	Fold change ApoE$^{-/-}$ ATLO vs. ApoE$^{-/-}$ adventitia no plaque	p ANOVA
1422124_a_at	Ptprc	protein tyrosine phosphatase, receptor type, C	19264	941	1071	6773	6.32	0.0004

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT adventitia	Mean ApoE$^{-/-}$ adventitia no plaque	Mean ApoE$^{-/-}$ ATLO	Fold change ApoE$^{-/-}$ ATLO vs. ApoE$^{-/-}$ adventitia no plaque	p ANOVA
1417876_at	Fcgr1	Fc receptor, IgG, high affinity I	14129	120	176	735	4.18	0.0004
1418340_at	Fcer1g	Fc receptor, IgE, high affinity I, gamma polypeptide	14127	435	682	2149	3.15	0.0004
1448620_at	Fcgr3	Fc receptor, IgG, low affinity III	14131	431	693	1955	2.82	0.001
1423954_at	C3	complement component 3	12266	5736	7932	18139	2.29	< 0.0001
1421775_at	Fcer1a	Fc receptor, IgE, high affinity I, alpha polypeptide	14125	52	202	134	-1.51	0.02
1424456_at	Pvrl2	poliovirus receptor-related 2	19294	177	287	138	-2.07	0.02

E) Positive regulation of B cell activation

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT adventitia	Mean ApoE$^{-/-}$ adventitia no plaque	Mean ApoE$^{-/-}$ ATLO	Fold change ApoE$^{-/-}$ ATLO vs. ApoE$^{-/-}$ adventitia no plaque	p ANOVA
1425324_x_at	Ighm	immunoglobulin heavy constant mu	16019	317	284	7656	26.93	0.04
1427869_at	Ighm	immunoglobulin heavy constant mu	16019	11	12	246	20.65	0.01
1427870_x_at	Ighm	immunoglobulin heavy constant mu	16019	1315	816	10203	12.50	0.05
1422124_a_at	Ptprc	protein tyrosine phosphatase, receptor type, C	19264	941	1071	6773	6.32	0.0004
1417976_at	Ada	adenosine deaminase	11486	68	66	354	5.36	0.008

Probe ID	Gene	Description	ID				Fold	p-value
1419307_at	Tnfrsf13c	tumor necrosis factor receptor superfamily, member 13c	72049	215	186	950	5.10	0.01
1417597_at	Cd28	CD28 antigen	12487	72	98	446	4.55	0.002
1416295_a_at	Il2rg	interleukin 2 receptor, gamma chain	16186	665	756	3396	4.49	0.001
1437270_a_at	Clcf1	cardiotrophin-like cytokine factor 1	56708	59	64	284	4.42	0.002
1427164_at	Il13ra1	interleukin 13 receptor, alpha 1	16164	52	61	268	4.40	0.0009
1460255_at	Tnfsf13b	tumor necrosis factor (ligand) superfamily, member 13b	24099	177	239	1045	4.38	0.0001
1428787_at	Nckap1l	NCK associated protein 1 like	105855	151	163	702	4.30	0.0007
1416296_at	Il2rg	interleukin 2 receptor, gamma chain	16186	401	329	1410	4.28	0.003
1425519_a_at	Cd74	CD74 antigen (invariant polypeptide of major histocompatibility complex, class II antigen-associated)	16149	3626	4458	18504	4.15	0.0002
1418261_at	Sykb	spleen tyrosine kinase	20963	307	392	1601	4.08	0.0002
1420425_at	Prdm1	PR domain containing 1, with ZNF domain	12142	126	110	402	3.66	0.002
1449473_s_at	Cd40	CD40 antigen	21939	86	105	376	3.58	0.0003
1418262_at	Sykb	spleen tyrosine kinase	20963	108	118	401	3.39	0.002
1427007_at	Sash3	SAM and SH3 domain containing 3	74131	394	423	1420	3.36	0.0004
1428786_at	Nckap1l	NCK associated protein 1 like	105855	289	404	1335	3.31	< 0.0001
1454783_at	Il13ra1	interleukin 13 receptor, alpha 1	16164	116	144	457	3.17	0.0008
1427329_a_at	Ighm	immunoglobulin heavy constant mu	16019	8628	9893	30659	3.10	0.03

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT adventitia	Mean ApoE$^{-/-}$ adventitia no plaque	Mean ApoE$^{-/-}$ ATLO	Fold change ApoE$^{-/-}$ ATLO vs. ApoE$^{-/-}$ adventitia no plaque	p ANOVA
1420351_at	Tnfrsf4	tumor necrosis factor receptor superfamily, member 4	22163	292	232	710	3.06	0.002
1433741_at	Cd38	CD38 antigen	12494	499	589	1580	2.68	0.0002
1418110_a_at	Inpp5d	inositol polyphosphate-5-phosphatase D	16331	362	433	821	1.90	0.002

F) B cell proliferation

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT adventitia	Mean ApoE$^{-/-}$ adventitia no plaque	Mean ApoE$^{-/-}$ ATLO	Fold change ApoE$^{-/-}$ ATLO vs. ApoE$^{-/-}$ adventitia no plaque	p ANOVA
1425324_x_at	Ighm	immunoglobulin heavy constant mu	16019	317	284	7656	26.93	0.04
1427869_at	Ighm	immunoglobulin heavy constant mu	16019	11	12	246	20.65	0.01
1427870_x_at	Ighm	immunoglobulin heavy constant mu	16019	1315	816	10203	12.50	0.05
1448576_at	Il7r	interleukin 7 receptor	16197	11	24	270	11.10	0.02
1448575_at	Il7r	interleukin 7 receptor	16197	140	168	1546	9.22	0.0002
1418830_at	Cd79a	CD79A antigen (immunoglobulin-associated alpha)	12518	229	158	1276	8.09	0.003
1423182_at	Tnfrsf13b	tumor necrosis factor receptor superfamily, member 13b	57916	143	130	959	7.38	0.002

1422124_a_at	Ptprc	protein tyrosine phosphatase, receptor type, C	19264	941	1071	6773	6.32	0.0004
1416034_at	Cd24a	CD24a antigen	12484	721	222	1324	5.95	0.02
1455332_x_at	Fcgr2b	Fc receptor, IgG, low affinity IIb	14130	252	390	2245	5.75	< 0.0001
1417976_at	Ada	adenosine deaminase	11486	68	66	354	5.36	0.008
1419307_at	Tnfrsf13c	tumor necrosis factor receptor superfamily, member 13c	72049	215	186	950	5.10	0.01
1451941_a_at	Fcgr2b	Fc receptor, IgG, low affinity IIb	14130	282	355	1782	5.01	0.0002
1435477_s_at	Fcgr2b	Fc receptor, IgG, low affinity IIb	14130	695	1072	5035	4.70	< 0.0001
1437270_a_at	Clcf1	cardiotrophin-like cytokine factor 1	56708	59	64	284	4.42	0.002
1427164_at	Il13ra1	interleukin 13 receptor, alpha 1	16164	52	61	268	4.40	0.0009
1460255_at	Tnfsf13b	tumor necrosis factor (ligand) superfamily, member 13b	24099	177	239	1045	4.38	0.0001
1448182_a_at	Cd24a	CD24a antigen	12484	2648	898	3859	4.30	0.004
1428787_at	Nckap1l	NCK associated protein 1 like	105855	151	163	702	4.30	0.0007
1425519_a_at	Cd74	CD74 antigen (invariant polypeptide of major histocompatibility complex, class II antigen-associated)	16149	3626	4458	18504	4.15	0.0002
1437502_x_at	Cd24a	CD24a antigen	12484	1303	540	2187	4.05	0.002
1435476_a_at	Fcgr2b	Fc receptor, IgG, low affinity IIb	14130	829	971	3572	3.68	< 0.0001
1420425_at	Prdm1	PR domain containing 1, with ZNF domain	12142	126	110	402	3.66	0.002
1449473_s_at	Cd40	CD40 antigen	21939	86	105	376	3.58	0.0003

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT adventitia	Mean ApoE$^{-/-}$ adventitia no plaque	Mean ApoE$^{-/-}$ ATLO	Fold change ApoE$^{-/-}$ ATLO vs. ApoE$^{-/-}$ adventitia no plaque	p ANOVA
1427007_at	Sash3	SAM and SH3 domain containing 3	74131	394	423	1420	3.36	0.0004
1428786_at	Nckap1l	NCK associated protein 1 like	105855	289	404	1335	3.31	< 0.0001
1454783_at	Il13ra1	interleukin 13 receptor, alpha 1	16164	116	144	457	3.17	0.0008
1427329_a_at	Ighm	immunoglobulin heavy constant mu	16019	8628	9893	30659	3.10	0.03
1420351_at	Tnfrsf4	tumor necrosis factor receptor superfamily, member 4	22163	292	232	710	3.06	0.002
1433741_at	Cd38	CD38 antigen	12494	499	589	1580	2.68	0.0002
1421205_at	Atm	ataxia telangiectasia mutated homolog (human)	11920	218	150	319	2.13	0.03
1422847_a_at	Prkcd	protein kinase C, delta	18753	496	489	1036	2.12	0.0005
1418110_a_at	Inpp5d	inositol polyphosphate-5-phosphatase D	16331	362	433	821	1.90	0.002

G) B cell differentiation

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT adventitia	Mean ApoE$^{-/-}$ adventitia no plaque	Mean ApoE$^{-/-}$ ATLO	Fold change ApoE$^{-/-}$ ATLO vs. ApoE$^{-/-}$ adventitia no plaque	p ANOVA
1425477_x_at	H2-Ab1	histocompatibility 2, class II antigen A, beta 1	14961	235	269	2613	9.70	0.0004
1460188_at	Ptpn6	protein tyrosine phosphatase, non-receptor type 6	15170	150	105	926	8.78	0.004

1418830_at	Cd79a	CD79A antigen (immunoglobulin-associated alpha)	12518	229	158	1276	8.09	0.003
1419406_a_at	Bcl11a	B cell CLL/lymphoma 11A (zinc finger protein)	14025	94	42	306	7.25	0.03
1422124_a_at	Ptprc	protein tyrosine phosphatase, receptor type, C	19264	941	1071	6773	6.32	0.0004
1451721_a_at	H2-Ab1	histocompatibility 2, class II antigen A, beta 1	14961	435	582	3528	6.06	0.0008
1416034_at	Cd24a	CD24a antigen	12484	721	222	1324	5.95	0.02
1417976_at	Ada	adenosine deaminase	11486	68	66	354	5.36	0.008
1419538_at	Flt3	FMS-like tyrosine kinase 3	14255	71	52	237	4.52	0.02
1416295_a_at	Il2rg	interleukin 2 receptor, gamma chain	16186	665	756	3396	4.49	0.001
1437270_a_at	Clcf1	cardiotrophin-like cytokine factor 1	56708	59	64	284	4.42	0.002
1448182_a_at	Cd24a	CD24a antigen	12484	2648	898	3859	4.30	0.004
1428787_at	Nckap1l	NCK associated protein 1 like	105855	151	163	702	4.30	0.0007
1416296_at	Il2rg	interleukin 2 receptor, gamma chain	16186	401	329	1410	4.28	0.003
1418261_at	Sykb	spleen tyrosine kinase	20963	307	392	1601	4.08	0.0002
1437502_x_at	Cd24a	CD24a antigen	12484	1303	540	2187	4.05	0.002
1450648_s_at	H2-Ab1	histocompatibility 2, class II antigen A, beta 1	14961	1634	1981	7773	3.92	0.0002
1420425_at	Prdm1	PR domain containing 1, with ZNF domain	12142	126	110	402	3.66	0.002
1418133_at	Bcl3	B cell leukemia/lymphoma 3	12051	96	165	570	3.45	0.001
1418262_at	Sykb	spleen tyrosine kinase	20963	108	118	401	3.39	0.002
1428786_at	Nckap1l	NCK associated protein 1 like	105855	289	404	1335	3.31	< 0.0001

1426926_at	Plcg2	phospholipase C, gamma 2	234779	494	418	1220	2.92	0.0002
1421141_a_at	Foxp1	forkhead box P1	108655	93	86	197	2.30	0.02
1421858_at	Adam17	a disintegrin and metallopeptidase domain 17	11491	429	477	1039	2.18	0.0003
1421205_at	Atm	ataxia telangiectasia mutated homolog (human)	11920	218	150	319	2.13	0.03
1456694_x_at	Ptpn6	protein tyrosine phosphatase, non-receptor type 6	15170	414	568	1084	1.91	0.004
1418110_a_at	Inpp5d	inositol polyphosphate-5-phosphatase D	16331	362	433	821	1.90	0.002
1435176_a_at	Id2	inhibitor of DNA binding 2	15902	1205	1571	2573	1.64	0.006
1421999_at	Tshr	thyroid stimulating hormone receptor	22095	213	278	122	-2.28	0.005

B2: Plaque-ATLO cluster: Differential expression of transcripts coding for B cell-regulating genes in WT and ApoE$^{-/-}$ aorta ATLO-plaque:

A) B cell mediated immunity

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean ApoE$^{-/-}$ plaque	Mean ApoE$^{-/-}$ ATLO	Fold change ApoE$^{-/-}$ ATLO vs. ApoE$^{-/-}$ plaque	p T-test of ApoE$^{-/-}$ ATLO vs. ApoE$^{-/-}$ plaque
1427756_x_at	Ighm	immunoglobulin heavy constant mu	16019	139	9012	64.79	0.009
1425324_x_at	Ighm	immunoglobulin heavy constant mu	16019	184	7656	41.51	0.01
1427869_at	Ighm	immunoglobulin heavy constant mu	16019	6	246	40.05	0.01

1416051_at	C2	complement component 2 (within H-2S)	12263	68	1652	24.25	0.02
1427870_x_at	Ighm	immunoglobulin heavy constant mu	16019	610	10203	16.71	0.01
1427329_a_at	Ighm	immunoglobulin heavy constant mu	16019	2675	30659	11.46	0.009
1425385_a_at	Ighm	immunoglobulin heavy constant mu	16019	1098	10938	9.96	0.04
1460242_at	Cd55	CD55 antigen	13136	56	321	5.75	0.01
1423954_at	C3	complement component 3	12266	3412	18139	5.32	0.002
1416625_at	Serping1	serine (or cysteine) peptidase inhibitor, clade G, member 1	12258	2789	13937	5.00	0.002
1418762_at	Cd55	CD55 antigen	13136	78	324	4.13	0.009
1418021_at	C4b	complement component 4B (Childo blood group)	12268	2810	10968	3.90	0.01
1449473_s_at	Cd40	CD40 antigen	21939	134	376	2.80	0.008
1419212_at	Icosl	icos ligand	50723	233	592	2.54	0.003
1427351_s_at	Ighm	immunoglobulin heavy constant mu	16019	9121	21660	2.37	0.02
1424041_s_at	C1s	complement component 1, s subcomponent	50908	1558	3663	2.35	0.003
1417009_at	C1ra	complement component 1, r subcomponent A	50909	923	2035	2.20	0.009
1434366_x_at	C1qb	complement component 1, q subcomponent, beta polypeptide	12260	19394	8974	-2.16	0.03
1417063_at	C1qb	complement component 1, q subcomponent, beta polypeptide	12260	11615	5054	-2.30	0.02
1417381_at	C1qa	complement component 1, q subcomponent, alpha polypeptide	12259	11634	4626	-2.51	0.02

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean ApoE$^{-/-}$ plaque	Mean ApoE$^{-/-}$ ATLO	Fold change ApoE$^{-/-}$ ATLO vs. ApoE$^{-/-}$ plaque	p T-test of ApoE$^{-/-}$ ATLO vs. ApoE$^{-/-}$ plaque
1448620_at	Fcgr3	Fc receptor, IgG, low affinity III	14131	5147	1955	-2.63	0.003
1418340_at	Fcer1g	Fc receptor, IgE, high affinity I, gamma polypeptide	14127	6206	2149	-2.89	0.003
1420653_at	Tgfb1	transforming groWTh factor, beta 1	21803	552	189	-2.91	0.04

B) B cell activation

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean ApoE$^{-/-}$ plaque	Mean ApoE$^{-/-}$ ATLO	Fold change ApoE$^{-/-}$ ATLO vs. ApoE$^{-/-}$ plaque	p T-test of ApoE$^{-/-}$ ATLO vs. ApoE$^{-/-}$ plaque
1427756_x_at	Ighm	immunoglobulin heavy constant mu	16019	139	9012	64.79	0.009
1425324_x_at	Ighm	immunoglobulin heavy constant mu	16019	184	7656	41.51	0.01
1427869_at	Ighm	immunoglobulin heavy constant mu	16019	6	246	40.05	0.01
1450912_at	Ms4a1	membrane-spanning 4-domains, subfamily A, member 1	12482	52	1147	22.21	0.009
1427870_x_at	Ighm	immunoglobulin heavy constant mu	16019	610	10203	16.71	0.01
1423226_at	Ms4a1	membrane-spanning 4-domains, subfamily A, member 1	12482	120	1842	15.41	0.01
1418830_at	Cd79a	CD79A antigen (immunoglobulin-associated alpha)	12518	92	1276	13.85	0.002
1427329_a_at	Ighm	immunoglobulin heavy constant mu	16019	2675	30659	11.46	0.009

1425385_a_at	Ighm	immunoglobulin heavy constant mu	16019	1098	10938	9.96	0.04
1419307_at	Tnfrsf13c	tumor necrosis factor receptor superfamily, member 13c	72049	107	950	8.92	0.005
1460255_at	Tnfsf13b	tumor necrosis factor (ligand) superfamily, member 13b	24099	182	1045	5.74	0.02
1422003_at	Cxcr5	chemokine (C-X-C motif) receptor 5	12145	52	294	5.68	0.01
1425289_a_at	Cr2	complement receptor 2	12902	86	394	4.58	0.03
1437502_x_at	Cd24a	CD24a antigen	12484	521	2187	4.19	0.005
1448182_a_at	Cd24a	CD24a antigen	12484	932	3859	4.14	0.009
1423182_at	Tnfrsf13b	tumor necrosis factor receptor superfamily, member 13b	57916	246	959	3.89	0.01
1417976_at	Ada	adenosine deaminase	11486	104	354	3.41	0.04
1449473_s_at	Cd40	CD40 antigen	21939	134	376	2.80	0.008
1420351_at	Tnfrsf4	tumor necrosis factor receptor superfamily, member 4	22163	267	710	2.66	0.005
1419212_at	Icosl	icos ligand	50723	233	592	2.54	0.003
1427351_s_at	Ighm	immunoglobulin heavy constant mu	16019	9121	21660	2.37	0.02
1426297_at	Tcf3	transcription factor 3	21423	205	444	2.16	0.03
1416295_a_at	Il2rg	interleukin 2 receptor, gamma chain	16186	1630	3396	2.08	0.04
1420425_at	Prdm1	PR domain containing 1, with ZNF domain	12142	201	402	2.00	0.03
1418163_at	Tlr4	toll-like receptor 4	21898	485	242	-2.00	0.04

1417122_at	Vav3	vav 3 oncogene	57257	429	196	-2.19	0.006
1426910_at	Pawr	PRKC, apoptosis, WT1, regulator	114774	1193	495	-2.41	0.01
1448575_at	Il7r	interleukin 7 receptor	16197	3839	1546	-2.48	0.01
1426169_a_at	Lat2	linker for activation of T cells family, member 2	56743	534	200	-2.67	0.007
1418162_at	Tlr4	toll-like receptor 4	21898	637	232	-2.74	0.003
1450136_at	Cd38	CD38 antigen	12494	432	153	-2.82	0.04
1422537_a_at	Id2	inhibitor of DNA binding 2	15902	1227	433	-2.83	0.002
1420653_at	Tgfb1	transforming groWTh factor, beta 1	21803	552	189	-2.91	0.04
1448576_at	Il7r	interleukin 7 receptor	16197	977	270	-3.62	0.01

C) Immunoglobulin

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean ApoE$^{-/-}$ plaque	Mean ApoE$^{-/-}$ ATLO	Fold change ApoE$^{-/-}$ ATLO vs. ApoE$^{-/-}$ plaque	p T-test of ApoE$^{-/-}$ ATLO vs. ApoE$^{-/-}$ plaque
1427756_x_at	Ighm	immunoglobulin heavy constant mu	16019	139	9012	64.79	0.009
1425324_x_at	Ighm	immunoglobulin heavy constant mu	16019	184	7656	41.51	0.01
1427869_at	Ighm	immunoglobulin heavy constant mu	16019	6	246	40.05	0.01

1424305_at	Igj	immunoglobulin joining chain	16069	1300	21748	16.73	0.003
1427329_a_at	Ighm	immunoglobulin heavy constant mu	16019	2675	30659	11.46	0.009
1425385_a_at	Ighm	immunoglobulin heavy constant mu	16019	1098	10938	9.96	0.04
1427351_s_at	Ighm	immunoglobulin heavy constant mu	16019	9121	21660	2.37	0.02

D) Positive regulation of B cell mediated immunity

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean $ApoE^{-/-}$ plaque	Mean $ApoE^{-/-}$ ATLO	Fold change $ApoE^{-/-}$ ATLO vs. $ApoE^{-/-}$ plaque	p T-test of $ApoE^{-/-}$ ATLO vs. $ApoE^{-/-}$ plaque
1423954_at	C3	complement component 3	12266	3412	18139	5.32	0.002
1448620_at	Fcgr3	Fc receptor, IgG, low affinity III	14131	5147	1955	-2.63	0.003
1418340_at	Fcer1g	Fc receptor, IgE, high affinity I, gamma polypeptide	14127	6206	2149	-2.89	0.003

E) Positive regulation of B cell activation

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean ApoE$^{-/-}$ plaque	Mean ApoE$^{-/-}$ ATLO	Fold change ApoE$^{-/-}$ ATLO vs. ApoE$^{-/-}$ plaque	p T-test of ApoE$^{-/-}$ ATLO vs. ApoE$^{-/-}$ plaque
1427756_x_at	Ighm	immunoglobulin heavy constant mu	16019	139	9012	64.79	0.009
1425324_x_at	Ighm	immunoglobulin heavy constant mu	16019	184	7656	41.51	0.01
1427869_at	Ighm	immunoglobulin heavy constant mu	16019	6	246	40.05	0.01
1427870_x_at	Ighm	immunoglobulin heavy constant mu	16019	610	10203	16.71	0.01
1427329_a_at	Ighm	immunoglobulin heavy constant mu	16019	2675	30659	11.46	0.009
1425385_a_at	Ighm	immunoglobulin heavy constant mu	16019	1098	10938	9.96	0.04
1419307_at	Tnfrsf13c	tumor necrosis factor receptor superfamily, member 13c	72049	107	950	8.92	0.005
1460255_at	Tnfsf13b	tumor necrosis factor (ligand) superfamily, member 13b	24099	182	1045	5.74	0.02
1417976_at	Ada	adenosine deaminase	11486	104	354	3.41	0.04
1449473_s_at	Cd40	CD40 antigen	21939	134	376	2.80	0.008
1420351_at	Tnfrsf4	tumor necrosis factor receptor superfamily, member 4	22163	267	710	2.66	0.005
1427351_s_at	Ighm	immunoglobulin heavy constant mu	16019	9121	21660	2.37	0.02

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean ApoE⁻/⁻ plaque	Mean ApoE⁻/⁻ ATLO	Fold change	p T-test
1426297_at	Tcf3	transcription factor 3	21423	205	444	2.16	0.03
1416295_a_at	Il2rg	interleukin 2 receptor, gamma chain	16186	1630	3396	2.08	0.04
1420425_at	Prdm1	PR domain containing 1, with ZNF domain	12142	201	402	2.00	0.03
1418163_at	Tlr4	toll-like receptor 4	21898	485	242	-2.00	0.04
1417122_at	Vav3	vav 3 oncogene	57257	429	196	-2.19	0.006
1418162_at	Tlr4	toll-like receptor 4	21898	637	232	-2.74	0.003
1450136_at	Cd38	CD38 antigen	12494	432	153	-2.82	0.04
1420653_at	Tgfb1	transforming groWTh factor, beta 1	21803	552	189	-2.91	0.04

F) B cell proliferation

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean ApoE⁻/⁻ plaque	Mean ApoE⁻/⁻ ATLO	Fold change ApoE⁻/⁻ ATLO vs. ApoE⁻/⁻ plaque	p T-test of ApoE⁻/⁻ ATLO vs. ApoE⁻/⁻ plaque
1427756_x_at	Ighm	immunoglobulin heavy constant mu	16019	139	9012	64.79	0.009
1425324_x_at	Ighm	immunoglobulin heavy constant mu	16019	184	7656	41.51	0.01
1427869_at	Ighm	immunoglobulin heavy constant mu	16019	6	246	40.05	0.01
1427870_x_at	Ighm	immunoglobulin heavy constant mu	16019	610	10203	16.71	0.01

1418830_at	Cd79a	CD79A antigen (immunoglobulin-associated alpha)	12518	92	1276	13.85	0.002
1427329_a_at	Ighm	immunoglobulin heavy constant mu	16019	2675	30659	11.46	0.009
1425385_a_at	Ighm	immunoglobulin heavy constant mu	16019	1098	10938	9.96	0.04
1419307_at	Tnfrsf13c	tumor necrosis factor receptor superfamily, member 13c	72049	107	950	8.92	0.005
1460255_at	Tnfsf13b	tumor necrosis factor (ligand) superfamily, member 13b	24099	182	1045	5.74	0.02
1437502_x_at	Cd24a	CD24a antigen	12484	521	2187	4.19	0.005
1448182_a_at	Cd24a	CD24a antigen	12484	932	3859	4.14	0.009
1423182_at	Tnfrsf13b	tumor necrosis factor receptor superfamily, member 13b	57916	246	959	3.89	0.01
1417976_at	Ada	adenosine deaminase	11486	104	354	3.41	0.04
1449473_s_at	Cd40	CD40 antigen	21939	134	376	2.80	0.008
1420351_at	Tnfrsf4	tumor necrosis factor receptor superfamily, member 4	22163	267	710	2.66	0.005
1427351_s_at	Ighm	immunoglobulin heavy constant mu	16019	9121	21660	2.37	0.02
1426297_at	Tcf3	transcription factor 3	21423	205	444	2.16	0.03
1420425_at	Prdm1	PR domain containing 1, with ZNF domain	12142	201	402	2.00	0.03
1418163_at	Tlr4	toll-like receptor 4	21898	485	242	-2.00	0.04
1417122_at	Vav3	vav 3 oncogene	57257	429	196	-2.19	0.006
1426910_at	Pawr	PRKC, apoptosis, WT1, regulator	114774	1193	495	-2.41	0.01

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean ApoE$^{-/-}$ plaque	Mean ApoE$^{-/-}$ ATLO	Fold change ApoE$^{-/-}$ ATLO vs. ApoE$^{-/-}$ plaque	p T-test of ApoE$^{-/-}$ ATLO vs. ApoE$^{-/-}$ plaque
1448575_at	Il7r	interleukin 7 receptor	16197	3839	1546	-2.48	0.01
1418162_at	Tlr4	toll-like receptor 4	21898	637	232	-2.74	0.003
1450136_at	Cd38	CD38 antigen	12494	432	153	-2.82	0.04
1448576_at	Il7r	interleukin 7 receptor	16197	977	270	-3.62	0.01

G) B cell differentiation

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean ApoE$^{-/-}$ plaque	Mean ApoE$^{-/-}$ ATLO	Fold change ApoE$^{-/-}$ ATLO vs. ApoE$^{-/-}$ plaque	p T-test of ApoE$^{-/-}$ ATLO vs. ApoE$^{-/-}$ plaque
1418830_at	Cd79a	CD79A antigen (immunoglobulin-associated alpha)	12518	92	1276	13.85	0.002
1437502_x_at	Cd24a	CD24a antigen	12484	521	2187	4.19	0.005
1448182_a_at	Cd24a	CD24a antigen	12484	932	3859	4.14	0.009
1417976_at	Ada	adenosine deaminase	11486	104	354	3.41	0.04
1426297_at	Tcf3	transcription factor 3	21423	205	444	2.16	0.03
1416295_a_at	Il2rg	interleukin 2 receptor, gamma chain	16186	1630	3396	2.08	0.04
1420425_at	Prdm1	PR domain containing 1, with ZNF domain	12142	201	402	2.00	0.03
1422537_a_at	Id2	inhibitor of DNA binding 2	15902	1227	433	-2.83	0.002

B3: Lymph node cluster: Differential expression of transcripts coding for B cell-regulating genes in WT and ApoE$^{-/-}$ renal lymph nodes (Fig.S2):

A) B cell mediated immunity

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT RLN	Mean ApoE$^{-/-}$ RLN	Mean ApoE$^{-/-}$ ATLO	Fold change ApoE$^{-/-}$ ATLO vs. ApoE$^{-/-}$ RLN	p ANOVA
1417876_at	Fcgr1	Fc receptor, IgG, high affinity I	14129	140	111	735	6.60	0.001
1434366_x_at	C1qb	complement component 1, q subcomponent, beta polypeptide	12260	2768	1852	8974	4.85	0.02
1437726_x_at	C1qb	complement component 1, q subcomponent, beta polypeptide	12260	3374	2557	10107	3.95	0.01
1448620_at	Fcgr3	Fc receptor, IgG, low affinity III	14131	463	499	1955	3.92	0.0006
1416625_at	Serping1	serine (or cysteine) peptidase inhibitor, clade G, member 1	12258	3642	3561	13937	3.91	0.002
1455332_x_at	Fcgr2b	Fc receptor, IgG, low affinity IIb	14130	714	649	2245	3.46	0.0009
1417063_at	C1qb	complement component 1, q subcomponent, beta polypeptide	12260	2116	1530	5054	3.30	0.008
1416051_at	C2	complement component 2 (within H-2S)	12263	560	648	1652	2.55	0.001
1417381_at	C1qa	complement component 1, q subcomponent, alpha	12259	2837	1821	4626	2.54	0.02

		polypeptide						
1435476_a_at	Fcgr2b	Fc receptor, IgG, low affinity IIb	14130	1530	1454	3572	2.46	0.001
1435477_s_at	Fcgr2b	Fc receptor, IgG, low affinity IIb	14130	2049	2053	5035	2.45	0.001
1451941_a_at	Fcgr2b	Fc receptor, IgG, low affinity IIb	14130	667	736	1782	2.42	0.01
1418021_at	C4b	complement component 4B (Childo blood group)	12268	4111	4530	10968	2.42	0.002
1424302_at	Lilrb3	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 3	18733	549	532	1245	2.34	0.01
1417009_at	C1ra	complement component 1, r subcomponent A	50909	827	1029	2035	1.98	0.007
1437271_at	Clcf1	cardiotrophin-like cytokine factor 1	56708	295	238	128	-1.85	0.02
1416915_at	Msh6	muts homolog 6 (E. coli)	17688	623	569	268	-2.12	0.008
1449361_at	Tbx21	T-box 21	57765	403	359	160	-2.24	0.005
1418026_at	Exo1	exonuclease 1	26909	185	148	63	-2.35	0.03
1449508_at	Il27ra	interleukin 27 receptor, alpha	50931	619	615	255	-2.41	0.002
1420353_at	Lta	lymphotoxin A	16992	348	282	115	-2.45	0.02
1460188_at	Ptpn6	protein tyrosine phosphatase, non-receptor type 6	15170	2532	2309	926	-2.49	0.02
1460415_a_at	Cd40	CD40 antigen	21939	701	556	212	-2.62	0.007
1423543_at	Swap70	SWA-70 protein	20947	2012	1857	655	-2.84	0.01
1417597_at	Cd28	CD28 antigen	12487	1260	1540	446	-3.45	0.003
1460242_at	Cd55	CD55 antigen	13136	1161	1111	321	-3.46	0.02

1418762_at	Cd55	CD55 antigen	13136	1018	1179	324	-3.63	0.005
1420577_at	Aicda	activation-induced cytidine deaminase	11628	304	249	68	-3.66	0.03
1437025_at	Cd28	CD28 antigen	12487	806	829	196	-4.23	0.0008

B) B cell activation

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT RLN	Mean ApoE$^{-/-}$ RLN	Mean ApoE$^{-/-}$ ATLO	Fold change ApoE$^{-/-}$ ATLO vs. ApoE$^{-/-}$ RLN	p ANOVA
1455332_x_at	Fcgr2b	Fc receptor, IgG, low affinity IIb	14130	714	649	2245	3.46	0.0009
1435476_a_at	Fcgr2b	Fc receptor, IgG, low affinity IIb	14130	1530	1454	3572	2.46	0.001
1435477_s_at	Fcgr2b	Fc receptor, IgG, low affinity IIb	14130	2049	2053	5035	2.45	0.001
1451941_a_at	Fcgr2b	Fc receptor, IgG, low affinity IIb	14130	667	736	1782	2.42	0.01
1424638_at	Cdkn1a	cyclin-dependent kinase inhibitor 1A (P21)	12575	398	384	860	2.24	0.05
1460255_at	Tnfsf13b	tumor necrosis factor (ligand) superfamily, member 13b	24099	453	470	1045	2.22	0.002
1434694_at	Lrrc8a	leucine rich repeat containing 8A	241296	456	450	987	2.20	0.005
1449839_at	Casp3	caspase 3	12367	1413	1148	666	-1.72	0.01
1437271_at	Clcf1	cardiotrophin-like cytokine factor 1	56708	295	238	128	-1.85	0.02

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT RLN	Mean ApoE$^{-/-}$ RLN	Mean ApoE$^{-/-}$ ATLO	Fold change ApoE$^{-/-}$ ATLO vs. ApoE$^{-/-}$ RLN	p ANOVA
1426297_at	Tcf3	transcription factor 3	21423	968	856	444	-1.93	0.004
1460419_a_at	Prkcb	protein kinase C, beta	18751	3332	3178	1599	-1.99	0.005
1420404_at	Cd86	CD86 antigen	12524	784	853	422	-2.02	0.002
1417372_a_at	Peli1	pellino 1	67245	2397	2213	1076	-2.06	0.004
1423478_at	Prkcb	protein kinase C, beta	18751	914	856	414	-2.07	0.02
1416915_at	Msh6	mutS homolog 6 (E. coli)	17688	623	569	268	-2.12	0.008
1453281_at	Pik3cd	phosphatidylinositol 3-kinase catalytic delta polypeptide	18707	1159	1199	565	-2.12	0.002
1427007_at	Sash3	SAM and SH3 domain containing 3	74131	3122	3033	1420	-2.14	0.002
1416335_at	Mif	macrophage migration inhibitory factor	17319	4803	4861	2252	-2.16	0.005
1421142_s_at	Foxp1	forkhead box P1	108655	738	723	333	-2.17	0.003
1421205_at	Atm	ataxia telangiectasia mutated homolog (human)	11920	682	707	319	-2.22	0.01
1449361_at	Tbx21	T-box 21	57765	403	359	160	-2.24	0.005
1455242_at	Foxp1	forkhead box P1	108655	1928	2227	986	-2.26	0.03
1451507_at	Mef2c	myocyte enhancer factor 2C	17260	645	583	255	-2.29	0.05

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT RLN	Mean ApoE$^{-/-}$ RLN	Mean ApoE$^{-/-}$ ATLO	Fold change ApoE$^{-/-}$ ATLO vs. ApoE$^{-/-}$ RLN	p ANOVA
1448575_at	Il7r	interleukin 7 receptor	16197	2453	3546	1546	-2.29	0.003
1418026_at	Exo1	exonuclease 1	26909	185	148	63	-2.35	0.03
1417371_at	Peli1	pellino 1	67245	1567	1680	705	-2.38	0.001
1449508_at	Il27ra	interleukin 27 receptor, alpha	50931	619	615	255	-2.41	0.002
1460188_at	Ptpn6	protein tyrosine phosphatase, non-receptor type 6	15170	2532	2309	926	-2.49	0.02
1460415_a_at	Cd40	CD40 antigen	21939	701	556	212	-2.62	0.007
1419307_at	Tnfrsf13c	tumor necrosis factor receptor superfamily, member 13c	72049	3033	2499	950	-2.63	0.01
1419406_a_at	Bcl11a	B cell CLL/lymphoma 11A (zinc finger protein)	14025	883	809	306	-2.64	0.03
1416296_at	Il2rg	interleukin 2 receptor, gamma chain	16186	3396	3793	1410	-2.69	0.003
1423543_at	Swap70	SWA-70 protein	20947	2012	1857	655	-2.84	0.01
1436861_at	Il7	interleukin 7	16196	359	369	129	-2.86	0.006
1421028_a_at	Mef2c	myocyte enhancer factor 2C	17260	691	763	262	-2.92	0.02
1421141_a_at	Foxp1	forkhead box P1	108655	487	581	197	-2.94	0.007
1419334_at	Ctla4	cytotoxic T-lymphocyte-associated protein 4	12477	488	472	147	-3.21	0.002

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT RLN	Mean ApoE$^{-/-}$ RLN	Mean ApoE$^{-/-}$ ATLO	Fold change ApoE$^{-/-}$ ATLO vs. ApoE$^{-/-}$ RLN	p ANOVA
1448576_at	Il7r	interleukin 7 receptor	16197	533	878	270	-3.25	0.02
1422003_at	Cxcr5	chemokine (C-X-C motif) receptor 5	12145	1241	986	294	-3.36	0.007
1450829_at	Tnfaip3	tumor necrosis factor, alpha-induced protein 3	21929	147	218	65	-3.38	0.03
1449858_at	Cd86	CD86 antigen	12524	396	507	149	-3.40	0.009
1417597_at	Cd28	CD28 antigen	12487	1260	1540	446	-3.45	0.003
1452389_at	Cd27	CD27 antigen	21940	444	489	138	-3.53	0.005
1450912_at	Ms4a1	membrane-spanning 4-domains, subfamily A, member 1	12482	4855	4060	1147	-3.54	0.04
1420577_at	Aicda	activation-induced cytidine deaminase	11628	304	249	68	-3.66	0.03
1437025_at	Cd28	CD28 antigen	12487	806	829	196	-4.23	0.0008
1421457_a_at	Samsn1	SAM domain, SH3 domain and nuclear localization signals, 1	67742	808	969	225	-4.30	0.004
1423226_at	Ms4a1	membrane-spanning 4-domains, subfamily A, member 1	12482	8776	7974	1842	-4.33	0.03
1422992_s_at	Pik3cd	phosphatidylinositol 3-kinase catalytic delta polypeptide	18707	250	285	48	-5.95	0.02
1425289_a_at	Cr2	complement receptor 2	12902	1524	2577	394	-6.54	0.02

C) Positive regulation of B cell mediated immunity

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT RLN	Mean ApoE$^{-/-}$ RLN	Mean ApoE$^{-/-}$ ATLO	Fold change ApoE$^{-/-}$ ATLO vs. ApoE$^{-/-}$ RLN	p ANOVA
1417876_at	Fcgr1	Fc receptor, IgG, high affinity I	14129	140	111	735	6.60	0.001
1448620_at	Fcgr3	Fc receptor, IgG, low affinity III	14131	463	499	1955	3.92	0.0006
1420353_at	Lta	lymphotoxin A	16992	348	282	115	-2.45	0.02

D) Positive regulation of B cell activation

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT RLN	Mean ApoE$^{-/-}$ RLN	Mean ApoE$^{-/-}$ ATLO	Fold change ApoE$^{-/-}$ ATLO vs. ApoE$^{-/-}$ RLN	p ANOVA
1424638_at	Cdkn1a	cyclin-dependent kinase inhibitor 1A (P21)	12575	398	384	860	2.24	0.05
1460255_at	Tnfsf13b	tumor necrosis factor (ligand) superfamily, member 13b	24099	453	470	1045	2.22	0.002

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT RLN	Mean ApoE$^{-/-}$ RLN	Mean ApoE$^{-/-}$ ATLO	Fold change ApoE$^{-/-}$ ATLO vs.	p ANOVA
1437271_at	Clcf1	cardiotrophin-like cytokine factor 1	56708	295	238	128	-1.85	0.02
1426297_at	Tcf3	transcription factor 3	21423	968	856	444	-1.93	0.004
1417372_a_at	Peli1	pellino 1	67245	2397	2213	1076	-2.06	0.004
1427007_at	Sash3	SAM and SH3 domain containing 3	74131	3122	3033	1420	-2.14	0.002
1416335_at	Mif	macrophage migration inhibitory factor	17319	4803	4861	2252	-2.16	0.005
1449361_at	Tbx21	T-box 21	57765	403	359	160	-2.24	0.005
1417371_at	Peli1	pellino 1	67245	1567	1680	705	-2.38	0.001
1460415_a_at	Cd40	CD40 antigen	21939	701	556	212	-2.62	0.007
1419307_at	Tnfrsf13c	tumor necrosis factor receptor superfamily, member 13c	72049	3033	2499	950	-2.63	0.01
1416296_at	Il2rg	interleukin 2 receptor, gamma chain	16186	3396	3793	1410	-2.69	0.003
1436861_at	Il7	interleukin 7	16196	359	369	129	-2.86	0.006
1417597_at	Cd28	CD28 antigen	12487	1260	1540	446	-3.45	0.003
1437025_at	Cd28	CD28 antigen	12487	806	829	196	-4.23	0.0008

E) B cell proliferation

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT RLN	Mean ApoE$^{-/-}$ RLN	Mean ApoE$^{-/-}$ ATLO	Fold change ApoE$^{-/-}$ ATLO vs.	p ANOVA

							ApoE$^{-/-}$ RLN	
1455332_x_at	Fcgr2b	Fc receptor, IgG, low affinity IIb	14130	714	649	2245	3.46	0.0009
1435476_a_at	Fcgr2b	Fc receptor, IgG, low affinity IIb	14130	1530	1454	3572	2.46	0.001
1435477_s_at	Fcgr2b	Fc receptor, IgG, low affinity IIb	14130	2049	2053	5035	2.45	0.001
1451941_a_at	Fcgr2b	Fc receptor, IgG, low affinity IIb	14130	667	736	1782	2.42	0.01
1424638_at	Cdkn1a	cyclin-dependent kinase inhibitor 1A (P21)	12575	398	384	860	2.24	0.05
1460255_at	Tnfsf13b	tumor necrosis factor (ligand) superfamily, member 13b	24099	453	470	1045	2.22	0.002
1449839_at	Casp3	caspase 3	12367	1413	1148	666	-1.72	0.01
1437271_at	Clcf1	cardiotrophin-like cytokine factor 1	56708	295	238	128	-1.85	0.02
1426297_at	Tcf3	transcription factor 3	21423	968	856	444	-1.93	0.004
1417372_a_at	Peli1	pellino 1	67245	2397	2213	1076	-2.06	0.004
1427007_at	Sash3	SAM and SH3 domain containing 3	74131	3122	3033	1420	-2.14	0.002
1416335_at	Mif	macrophage migration inhibitory factor	17319	4803	4861	2252	-2.16	0.005
1421205_at	Atm	ataxia telangiectasia mutated homolog (human)	11920	682	707	319	-2.22	0.01
1451507_at	Mef2c	myocyte enhancer factor 2C	17260	645	583	255	-2.29	0.05
1448575_at	Il7r	interleukin 7 receptor	16197	2453	3546	1546	-2.29	0.003
1417371_at	Peli1	pellino 1	67245	1567	1680	705	-2.38	0.001
1460415_a_at	Cd40	CD40 antigen	21939	701	556	212	-2.62	0.007

1419307_at	Tnfrsf13c	tumor necrosis factor receptor superfamily, member 13c	72049	3033	2499	950	-2.63	0.01
1436861_at	Il7	interleukin 7	16196	359	369	129	-2.86	0.006
1421028_a_at	Mef2c	myocyte enhancer factor 2C	17260	691	763	262	-2.92	0.02
1419334_at	Ctla4	cytotoxic T-lymphocyte-associated protein 4	12477	488	472	147	-3.21	0.002
1448576_at	Il7r	interleukin 7 receptor	16197	533	878	270	-3.25	0.02
1452389_at	Cd27	CD27 antigen	21940	444	489	138	-3.53	0.005

F) B cell differentiation

Affymetrix Probe set ID	Gene symbol	Gene name	Entrez Gene ID	Mean WT RLN	Mean ApoE$^{-/-}$ RLN	Mean ApoE$^{-/-}$ ATLO	Fold change ApoE$^{-/-}$ ATLO vs. ApoE$^{-/-}$ RLN	p ANOVA
1434694_at	Lrrc8a	leucine rich repeat containing 8A	241296	456	450	987	2.20	0.005
1437271_at	Clcf1	cardiotrophin-like cytokine factor 1	56708	295	238	128	-1.85	0.02
1426297_at	Tcf3	transcription factor 3	21423	968	856	444	-1.93	0.004
1421142_s_at	Foxp1	forkhead box P1	108655	738	723	333	-2.17	0.003
1421205_at	Atm	ataxia telangiectasia mutated homolog (human)	11920	682	707	319	-2.22	0.01
1455242_at	Foxp1	forkhead box P1	108655	1928	2227	986	-2.26	0.03

1460188_at	Ptpn6	protein tyrosine phosphatase, non-receptor type 6	15170	2532	2309	926	-2.49	0.02
1419406_a_at	Bcl11a	B cell CLL/lymphoma 11A (zinc finger protein)	14025	883	809	306	-2.64	0.03
1416296_at	Il2rg	interleukin 2 receptor, gamma chain	16186	3396	3793	1410	-2.69	0.003
1421141_a_at	Foxp1	forkhead box P1	108655	487	581	197	-2.94	0.007
1450829_at	Tnfaip3	tumor necrosis factor, alpha-induced protein 3	21929	147	218	65	-3.38	0.03

Summary table I. Detailed mRNA profiles of spleen, blood, lymph nodes and aorta of WT and ApoE$^{-/-}$ mice.

(A) The tables indicate differential gene expression profiles in WT and ApoE$^{-/-}$ aorta **(A1)**, spleen **(A2)** and blood **(A3)**.

(B) The tables indicate differential gene expression profiles of indicated adventitia cluster **(B1)**, plaque-ATLO cluster **(B2),** and lymph node cluster **(B3)** for GO terms: B cell mediated immunity, B cell activation, immunoglobulin, positive regulation of B cell mediated immunity, positive regulation of B cell activation, B cell proliferation, and B cell differentiation. One way ANOVA or student's t test was applied.