SUPPLEMENTAL MATERIAL

LDL-reactive T cells regulate plasma cholesterol levels and development of atherosclerosis in humanized hypercholesterolemic mice

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**Supplemental Methods**
List of antibodies used for flow cytometry analysis.
Figure S1. Phenotype of BT transgenic mice.
(A) Experimental set up for phenotypic analysis.
(B) IFN-γ in supernatant after stimulation of splenocytes with 10 µg/ml human LDL, 10 µg/ml human ApoB100, and anti-CD3/anti-CD28, measured by ELISA (B6 n=6, BT1 n=4, 2-way ANOVA with Bonferroni’s post test).
(C) BT1 splenocytes incubated with native LDL or oxLDL for 60 hours in vitro (n=4, 2-way ANOVA with Bonferroni’s post test, dots show mean ± SEM).
(D) Percentage of CD3⁺CD4⁺ T-helper cells in the spleen (B6 n=8, BT1 n=8, BT2 n=4, BT3 n=8).
(E) TRBV31⁺ T-helper cells in the spleen (B6 n=9, BT1 n=9, BT2 n=7, BT3 n=11).
(F) Naïve CD62L⁺ cells in the TRBV31⁺ T-helper cell population in the spleen.
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(H) Foxp3⁺ regulatory T cells in the TRBV31⁺ T-helper cell population in the spleen.
(I) Experimental design of LDL injection into Nur77-GFP reporter mice.
(J) Nur77-GFP expression in TRBV31⁺ T-helper cells (Negative control n=2, BT1 n=4, BT2 n=3, BT3 n=3, Positive control n=2). Uninjected Nur77-GFP⁺ mice were used as negative controls (-). Splenocytes from Nur77-GFP⁺ mice treated with anti-CD3 and anti-CD28 in vitro overnight were used as positive controls (+).
(K) CD69 expression in TRBV31⁺ T-helper cells after LDL injection.
(L) CD25 expression in TRBV31⁺ T-helper cells after LDL injection.
Figure S2. CD4\(^+\) BT-cell proliferation in HuBL mice.

(A) Experimental design of labelled CD4\(^+\) cell transfer into HuBL mice.
(B) Flow cytometry plots showing proliferation of CD4\(^+\) T-helper cells labelled with CellTraceViolet transferred to HuBL recipients that expresses the antigen for BT1 cells. The upper panel shows splenic T-helper cells 1-3 days after the transfer of CD4\(^+\) T cells from B6 mice, and the lower panel shows transfer of CD4\(^+\) cells from BT1 mice 1-4 days after the transfer.
(C) The relative number of CellTraceViolet-labelled CD4\(^+\) T cells from B6 and BT1 mice in different organs 1-4 days after the transfer into HuBL mice.
(D) Purity of the isolated CD4\(^+\) cells being transferred after negative selection (B6 \(n=9\), BT1 \(n=9\), BT2 \(n=7\), BT3 \(n=11\)).
(E) Plots showing proliferation of BT1 CD4\(^+\) T cells transferred into HuBL mice with staining of Foxp3 on the y-axis. No induction of regulatory T cells was seen during this initial response to the antigen.
(F) Histogram showing proliferation of CellTraceViolet-labelled BT2 CD4\(^+\) T cells injected into an Ldlr\(^-\) recipient, without the antigen (gray line), and a HuBL recipient, with transgenic supply of human ApoB (black line). The samples are gated on CD3\(^+\)CD4\(^+\)CellTraceViolet\(^+\) T-helper cells in the inguinal lymph nodes 72 hours after injection. The x-axis shows fluorescence intensity and the y-axis the number of events.
Figure S3. Cell populations in spleens of *HuBL* mice injected with CD4⁺ BT cells.

(A) Total splenocyte count after meshing the organ through a cell strainer (*HuBL*/B6 n=15, *HuBL*/BT1 n=12, *HuBL*/BT2 n=10, *HuBL*/BT3 n=11).

(A-N) 1-way ANOVA with Dunnett’s post test, dots represent individual mice, bars show mean ± SEM.

(B-C) CD3⁺ CD4⁺ T-helper cells.

(B-N) *HuBL*/B6 n=11, *HuBL*/BT1 n=7, *HuBL*/BT2 n=10, *HuBL*/BT3 n=11.

(D) CD62L⁻ CD44⁺ naïve T-helper cells.

(E) CD44⁺ CD62L⁻ effector/memory T-helper cells.

(F) TRBV31⁺ T-helper cells.

(G) Tbet⁺ TRBV31⁺ Th1 cells.

(H) Foxp3⁺ TRBV31⁺ regulatory T-helper cells.

(I-J) PD1⁺ CXCR5⁺ CD44⁺ CD62L⁻ Tfh cells.

(K) CD19⁺ B220<sup>low</sup> B1 cells.

(L) CD19⁺ B220<sup>high</sup> B2 cells.

(M) GL7⁺ CD95⁺ IgD<sup>low</sup> germinal center B cells.

(N) CD138⁺ CD28⁺ plasma cells.

(O) Immunofluorescence micrographs of spleen showing B cells (B220⁺, green) and plasma cells (CD138⁺, blue), with a 100 µm scale bar.
Figure S4. Antibodies and lipid distribution in HuBL mice injected with CD4+ BT cells. (A-B) Anti-native LDL IgM and IgG. (A-J) Graphs show mean ± SEM, titers are displayed on the x-axis, and the y-axis shows optical density at 450 nm. (HuBL/B6 n=12, HuBL/BT1 n=11, HuBL/BT2 n=10, HuBL/BT3 n=10, 2-way ANOVA with Bonferroni’s post test, ns=not significant). (C-D) Anti-oxLDL IgM and IgG. (E-H) Anti-ApoB100 IgM, IgG, IgG1, and IgG2c. (I-J) Immune complexes between LDL and IgM or IgG, respectively. (K) Micrographs of Oil Red O-staining in glomeruli of kidneys, with a 50 µm scale bar. Nuclei counterstained with hematoxylin. (L) Immunofluorescence micrographs showing IgG1 (green), C3 (red), and nuclei (DAPI+, blue) in glomeruli of kidneys, with a 50 µm scale bar. (M) Immunofluorescence micrographs showing IgG2c (green), C3 (red), and nuclei (DAPI+, blue) in glomeruli of kidneys, with a 50 µm scale bar. (N) Fluorescent micrographs showing lipids (Nile Red+, pink) and macrophages (F4/80+, blue) in spleen, with a 100 µm scale bar.
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(O) Cholesterol content in liver tissue extracts (HuBL/B6 n=14, HuBL/BT1 n=12, HuBL/BT2 n=9, HuBL/BT3 n=12, Kruskal-Wallis test with Dunn’s post test).
Figure S5. T-helper cell phenotype and antibodies in BT1xHuBL mice.

(A) CD4^+CD8^− double negative, CD4^+CD8^+ double positive, CD4 single positive, and CD8 single positive TRBV31^+ thymocytes (HuBL n=3, BT1xHuBL n=3, BT1 n=3, 2-way ANOVA with Bonferroni’s post test).

(A-H) Dots represent individual mice, bars show mean ± SEM.

(B) Proportion of TRBV31^{bright} T-helper cells among CD4^+ T cells in the spleen (HuBL n=10, BT1xHuBL n=13, Student’s t-test).

(C) TRBV31^{dim} T-helper cells (HuBL n=10, BT1xHuBL n=13, Student’s t-test).

(D) Correlation between Trav12 and Trbv31 mRNA levels in para-aortic lymph nodes in BT1xHuBL mice (n=12, Pearson correlation).

(E) Correlation between Trav12 and Trbv31 mRNA levels in para-aortic lymph nodes in HuBL mice (n=10, Pearson correlation).

(F) CD62L-naïve T-helper cells, the circles show the TRBV31^{bright} population and the closed diamonds the TRBV31^{dim} population that is missing in the HuBL mice as shown in C (HuBL n=10, BT1xHuBL n=11, 1-way ANOVA with Bonferroni’s post test).

(G) Tbet^{+}TRBV31^{+} T-helper cells (HuBL n=9, BT1xHuBL n=10, 1-way ANOVA with Bonferroni’s post test).

(H) Foxp3^{+} regulatory T-helper cells (HuBL n=10, BT1xHuBL n=13, 1-way ANOVA with Dunnett’s post test).

(I-J) Anti-native LDL IgM and IgG.

(I-R) Graphs show mean ± SEM, titers are displayed on the x-axis, and the y-axis shows optical density at 450 nm.
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(HuBL n=10, BT1xHuBL n=12, 2-way ANOVA with Bonferroni’s post test).

(K-L) Anti-oxLDL IgM and IgG.

(M-P) Anti-ApoB100 IgM, IgG, IgG1, and IgG2c.

(Q-R) Immune complexes between LDL and IgM or IgG, respectively.

(S-T) Lipoprotein cholesterol and triglyceride profiles (HuBL n=5, HuBLxBT1 n=5, 2-way ANOVA with Bonferroni’s post test, curve shows mean values).

(U) Correlation between plasma cholesterol and LDL-IgG immune complexes in BT1xHuBL mice (n=12, Pearson correlation).
Figure S6. Lipid distribution and atherosclerotic plaque composition in BT1xHuBL mice.
(A) Immunofluorescence micrographs showing human ApoB (green) and nuclei (DAPI+, blue) in duodenum, with a 100 µm scale bar.
(B) Immunofluorescence micrographs showing human ApoB (green) and nuclei (DAPI+, blue) in liver, with a 100 µm scale bar.
(C) Micrographs of Oil Red O-stained liver sections with a 500 µm scale bar.
(D) Cholesterol content in liver tissue extracts (HuBL n=10, BT1xHuBL n=15, Mann-Whitney test).
(D-E, G-J, and L-O) Dots represent individual mice, bars show mean ± SEM.
(E) Lesions in the innominate artery analyzed in en face preparations (HuBL n=8, BT1xHuBL n=9, Student’s t-test).
(F) Quantification of the Oil Red O-stained lesion area in eight consecutive sections, 100 – 800 µm from the aortic root (HuBL n=7, BT1xHuBL n=8, 2-way ANOVA with Bonferroni’s post test, braces indicate significance level for strain comparison).
(G) Quantification of immunohistochemical staining of CD4+ T cells in atherosclerotic lesions in aortic root sections.
(H) Quantification of immunohistochemical staining of CD8+ T cells in atherosclerotic lesions in aortic root sections.
(I) Quantification of CD68+ stained area in atherosclerotic lesions in aortic root sections.
(J) Quantification of VCAM-1+ stained area in atherosclerotic lesions in aortic root sections.
(K) Micrographs of VCAM-1 stained (brown) atherosclerotic lesions in the aortic root with a 100 µm scale bar, dotted lines delineate lesion area. Hematoxylin counterstaining visualizes nuclei (blue).
(L) Quantification of immunohistochemical staining of IAα+ cells in atherosclerotic lesions in aortic root sections.
(M) Quantification of α-SM-actin+ stained area in atherosclerotic lesions in aortic root sections.
(N) Correlation between lesions in innominate artery and plasma cholesterol in BT1xHuBL mice (n=12, Pearson correlation).
(O) Correlation between lesions in innominate artery and LDL-IgG immune complexes in BT1xHuBL mice (n=9, Pearson correlation).
Figure S7. Phenotype of ApoB100-vaccinated HuBL mice.

(A) Body weight (HuBL mice; PBS-adjuvant n=6, ApoB100-adjuvant n=5, triangles represent individual mice, bars show mean ± SEM).

(B-D) Anti-ApoB100 IgM, IgG1, and IgG2c. Graphs show mean ± SEM, titers are displayed on the x-axis, and the y-axis shows optical density at 450 nm (HuBL mice; PBS-adjuvant n=6, ApoB100-adjuvant n=5, 2-way ANOVA with Bonferroni’s post test).

(E) Quantification of Oil Red O-stained lesion area in eight consecutive sections, 100 – 800 µm from the aortic root (HuBL mice; PBS-adjuvant n=5, ApoB100-adjuvant n=4, 2-way ANOVA with Bonferroni’s post test, graph shows mean ± SEM, braces indicate significance level for treatment comparison).
Figure S8. Characterization of BT3xHuBL mice.

(A) Number of cells in thymus counted after preparation of single cell suspension (HuBL n=5, BT3xHuBL n=9, BT3 n=2, 1-way ANOVA with Bonferroni’s post test).

(B) Representative flow cytometry plots showing expression of CD4 and CD8 in thymocytes.

(C) Design of diet experiment with compound mutant mice and purified IgG transfer experiment.

(D) CD3+CD4+ T-helper cells in spleen (HuBL n=16, BT3xHuBL n=12, Mann-Whitney test).

(E) Proportion CD44+CD62L- effector/memory of T-helper cells in spleen (HuBL n=11, BT3xHuBL n=12, Student’s t-test).

(F) Proportion Tbet+ Th1 of TRBV31+ T-helper cells in spleen (HuBL n=16, BT3xHuBL n=7, Mann-Whitney test).

(G) Proportion Foxp3+ T regulatory cells of TRBV31+ T-helper cells in spleen (HuBL n=16, BT3xHuBL n=7, Student’s t-test).

(H) Anti-native LDL IgM and IgG in plasma.

(I) Anti-oxLDL IgM and IgG in plasma.

(J) Anti-ApoB100 IgM, IgG, IgG1, and IgG2c in plasma.

(K) Competition assay for evaluation of anti-LDL IgG specificity in total IgG isolated from HuBL mice (n=4, 2-way ANOVA with Bonferroni’s post test).

(L) Anti-ApoB100 IgM, IgG, IgG1, and IgG2c in plasma.

(M) Anti-oxLDL IgM and IgG in plasma.

(N) Anti-ApoB100 IgM, IgG, IgG1, and IgG2c in plasma.

(O) Anti-oxLDL IgM and IgG in plasma.

(P) Competition assay for evaluation of anti-LDL IgG specificity in total IgG isolated from HuBL mice (n=4, 2-way ANOVA with Bonferroni’s post test).
ANOV A with Bonferroni’s post test, significant competition by all three competitors).

(Q) Competition assay for evaluation of anti-oxLDL IgG specificity in total IgG isolated from HuBL mice (n=4, 2-way ANOVA with Bonferroni’s post test, significant competition by all three competitors).

(R-S) Immune complexes between LDL and IgM or IgG, respectively.

(T) Quantification of average Oil Red O+ lipid droplet size in liver sections (HuBL n=9, BT3xHuBL n=5, Mann-Whitney test).

(U) Quantification of IgG1+ stained area in liver sections (HuBL n=13, BT3xHuBL n=6, Student’s t-test).

(V) Immunofluorescence micrographs showing human ApoB (green) and nuclei (DAPI+, blue) in liver, with a 100 µm scale bar.

(X) Immunofluorescence micrographs showing human ApoB (green) and nuclei (DAPI+, blue) in duodenum, with a 100 µm scale bar.
Table S1. Phenotype of HuBL mice injected with CD4+ BT cells*

|                          | HuBL/B6     | HuBL/BT1    | HuBL/BT2    | HuBL/BT3    |
|--------------------------|-------------|-------------|-------------|-------------|
| **Body weight (g)**      | 28.5 ± 0.7 n=15 | 30.5 ± 0.9 n=12 | 26.3 ± 0.6 n=10 | 28.1 ± 1.4 n=12 |
| **Plasma ApoB† (g/l)**   | 7.35 ± 0.44 n=7 | 5.86 ± 0.26 n=8 | 6.43 ± 0.69 n=10 | 4.30 ± 0.60 n=5 |
| **Plasma Cholesterol (mmol/l)** | 45.4 ± 3.1 n=14 | 34.3 ± 3.0 n=11 | 35.2 ± 6.4 n=9 | 28.0 ± 2.6 n=11 |
| **Plasma Triglycerides (mmol/l)** | 8.62 ± 0.62 n=14 | 8.99 ± 0.73 n=11 | 7.54 ± 0.89 n=9 | 6.40 ± 0.71 n=11 |
| **Plasma Creatinine (µmol/l)** | 88.9 ± 16.3 n=12 | 64.2 ± 14.7 n=8 | 70.7 ± 10.0 n=9 | 57.3 ± 11.0 n=9 |
| **Blood WBC‡ (10⁹/l)**   | 12.1 ± 0.9 n=15 | 12.2 ± 1.6 n=12 | 13.8 ± 1.6 n=10 | 14.2 ± 1.6 n=12 |
| **Blood Lymphocytes (10⁶/l)** | 7.56 ± 0.35 n=15 | 7.83 ± 0.52 n=12 | 9.59 ± 0.73 n=10 | 10.1 ± 0.78 n=12 |
| **Blood Monocytes (10⁶/l)** | 0.47 ± 0.06 n=15 | 0.43 ± 0.04 n=12 | 0.49 ± 0.06 n=10 | 0.54 ± 0.06 n=12 |
| **Blood Granulocytes (10⁶/l)** | 4.10 ± 0.61 n=15 | 3.91 ± 0.34 n=12 | 3.71 ± 0.38 n=10 | 3.57 ± 0.47 n=12 |
| **Spleen Lymphocytes (10⁶)** | 117 ± 9.5 n=15 | 151 ± 12 n=12 | 146 ± 14 n=10 | 241 ± 26 n=12 |
| **Spleen Monocytes (10⁶)** | 3.58 ± 0.56 n=15 | 4.17 ± 0.52 n=12 | 4.00 ± 0.60 n=10 | 8.97 ± 1.5 n=12 |
| **Spleen Granulocytes (10⁶)** | 15.3 ± 2.4 n=15 | 20.7 ± 2.3 n=12 | 19.4 ± 2.8 n=10 | 37.3 ± 6.5 n=12 |

* After a western diet period of 10 weeks
† After a western diet period of five weeks.
‡ WBC=White blood cells
Table S2. mRNA levels in organs of HuBL mice injected with CD4+ BT cells*

| Organ        | mRNA | HuBL/B6   | HuBL/BT1  | HuBL/BT2  | HuBL/BT3  |
|--------------|------|-----------|-----------|-----------|-----------|
| Aorta        | Trb31| 1.12 ± 0.15 n=14 | 3.08 ± 0.61 n=12 | 1.77 ± 0.22 n=9 | 15.1§ ± 2.0 n=10 |
|              | Tbx21| 1.14 ± 0.17 n=14 | 1.71 ± 0.23 n=10 | 1.04 ± 0.12 n=9 | 2.68‡ ± 0.35 n=10 |
|              | Gata3| 1.09 ± 0.12 n=14 | 1.28 ± 0.16 n=10 | 0.88 ± 0.09 n=9 | 1.21 ± 0.25 n=10 |
|              | Rorc | 1.38 ± 0.33 n=14 | 1.77 ± 0.31 n=10 | 0.90 ± 0.15 n=9 | 0.83 ± 0.17 n=10 |
|              | Foxp3| 1.20 ± 0.20 n=14 | 4.23† ± 0.06 n=10 | 4.33‡ ± 0.74 n=9 | 9.97§ ± 1.89 n=10 |
|              | Il6  | 1.23 ± 0.22 n=14 | 1.38 ± 0.28 n=12 | 1.39 ± 0.21 n=6 | 2.68 ± 0.82 n=8  |
|              | Il10 | 1.11 ± 0.13 n=14 | 1.82 ± 0.24 n=10 | 1.22 ± 0.23 n=9 | 2.35§ ± 0.20 n=10 |
|              | Ifng | 1.15 ± 0.18 n=14 | 2.60† ± 0.44 n=10 | 1.45 ± 0.20 n=9 | 12.5§ ± 3.13 n=10 |
|              | Il21 | 0.71 ± 0.46 n=14 | 5.91 ± 2.14 n=10 | 5.01 ± 1.23 n=9 | 36.4§ ± 13.7 n=10 |
| Para-aortic LN|| Trb31| 1.67 ± 0.43 n=14 | 3.20† ± 0.53 n=12 | 1.42 ± 0.26 n=10 | 2.57 ± 0.44 n=12 |
|              | Tbx21| 1.14 ± 0.18 n=14 | 1.37 ± 0.16 n=12 | 1.24 ± 0.18 n=10 | 1.28 ± 0.20 n=12 |
|              | Gata3| 1.19 ± 0.20 n=14 | 0.73 ± 0.10 n=12 | 0.80 ± 0.16 n=10 | 0.59† ± 0.06 n=12 |
|              | Rorc | 1.17 ± 0.16 n=14 | 0.83 ± 0.07 n=12 | 0.69 ± 0.09 n=10 | 0.78† ± 0.24 n=12 |
|              | Foxp3| 1.16 ± 0.16 n=14 | 1.10 ± 0.13 n=12 | 1.30 ± 0.21 n=10 | 1.20 ± 0.17 n=12 |
|              | Il4  | 1.12 ± 0.12 n=14 | 8.45§ ± 1.14 n=12 | 6.67‡ ± 0.98 n=10 | 9.69§ ± 1.70 n=12 |
|              | Il10 | 1.27 ± 0.27 n=14 | 1.45 ± 0.26 n=12 | 0.74 ± 0.17 n=10 | 0.88 ± 0.13 n=12 |
|              | Ifng | 1.29 ± 0.27 n=14 | 2.56† ± 0.38 n=12 | 1.28 ± 0.17 n=10 | 2.49‡ ± 0.35 n=12 |
|              | Il21 | 1.12 ± 0.13 n=14 | 6.02‡ ± 1.10 n=12 | 9.36§ ± 1.93 n=10 | 12.8§ ± 2.05 n=12 |
| Liver        | APOB | 0.99 ± 0.10 n=13 | 0.92 ± 0.07 n=12 | 1.03 ± 0.10 n=10 | 0.80 ± 0.07 n=12 |
|              | Col1a1| 1.56 ± 0.42 n=14 | 0.69 ± 0.15 n=12 | 1.88 ± 0.75 n=10 | 1.95 ± 0.71 n=11 |
|              | Col3a1| 1.57 ± 0.45 n=14 | 1.46 ± 0.59 n=12 | 1.54 ± 0.35 n=9 | 2.67 ± 0.75 n=11 |
|              | Tnf  | 1.65 ± 0.59 n=14 | 0.49 ± 0.07 n=12 | 2.77 ± 0.91 n=10 | 1.76 ± 0.54 n=11 |
|              | Tgfb1| 1.43 ± 0.31 n=14 | 1.17 ± 0.30 n=12 | 1.75 ± 0.45 n=9 | 1.82 ± 0.38 n=12 |

* Data calculated as relative expression using the 2^(-ΔΔCt) formula, normalized to the house-keeping gene Hprt
† p ≤ .05, Kruskal-Wallis test with Dunn’s multiple comparison test vs. HuBL/B6 group
‡ p ≤ .01, Kruskal-Wallis test with Dunn’s multiple comparison test vs. HuBL/B6 group
§ p ≤ .001, Kruskal-Wallis test with Dunn’s multiple comparison test vs. HuBL/B6 group
|| LN=lymph nodes
Table S3. Phenotype of HuBL vs. BT1xHuBL mice*

|                        | HuBL          | BT1xHuBL      | Significance |
|------------------------|---------------|---------------|--------------|
|                        | *          |               | (Student’s t-test) |
| **Body weight** (g)   | 32.0 ± 0.5 n=10 | 31.3 ± 0.7 n=13 | n.s.†         |
| **Blood WBC‡** (10⁹/l) | 12.2 ± 0.9 n=10 | 12.4 ± 1.6 n=13 | n.s.          |
| **Blood Lymphocytes** (10⁹/l) | 8.21 ± 0.60 n=10 | 8.20 ± 1.07 n=13 | n.s.          |
| **Blood Monocytes** (10⁹/l) | 0.42 ± 0.04 n=10 | 0.45 ± 0.07 n=13 | n.s.          |
| **Blood Granulocytes** (10⁹/l) | 3.55 ± 0.33 n=10 | 3.80 ± 0.54 n=13 | n.s.          |
| **Plasma Creatinine** (µmol/l) | 84.7 ± 11.5 n=14 | 92.2 ± 8.45 n=15 | n.s.          |

* After a western diet period of 10 weeks  
† n.s.=not significant  
‡ WBC=White blood cells
| Organ          | mRNA | HuBL        | BT1xHuBL  | Significance (Mann-Whitney test) |
|---------------|------|-------------|-----------|----------------------------------|
| Aorta         | Trbv31 | 1.44 ± 0.39 n=10 | 4.50 ± 0.24 n=11 | p = .0003                        |
|               | Tbx21  | 1.16 ± 0.24 n=10 | 0.99 ± 0.13 n=11 | n.s.                             |
|               | Gata3  | 1.09 ± 0.15 n=10 | 1.19 ± 0.12 n=11 | n.s.                             |
|               | Rorc   | 1.06 ± 0.12 n=10 | 1.13 ± 0.24 n=11 | n.s.                             |
|               | Foxp3  | 1.11 ± 0.18 n=10 | 0.66 ± 0.05 n=10 | p = .02                          |
|               | Il5    | 1.06 ± 0.10 n=10 | 1.15 ± 0.09 n=11 | n.s.                             |
|               | Il6    | 1.29 ± 0.24 n=10 | 1.19 ± 0.18 n=11 | n.s.                             |
|               | Il10   | 1.06 ± 0.24 n=10 | 0.68 ± 0.05 n=11 | p = .01                          |
|               | Foxp3  | 1.04 ± 0.11 n=10 | 0.88 ± 0.07 n=12 | n.s.                             |
|               | Il5    | 1.05 ± 0.11 n=10 | 1.69 ± 0.52 n=12 | n.s.                             |
|               | Il6    | 1.11 ± 0.21 n=10 | 0.71 ± 0.04 n=12 | p = .02                          |
|               | Il10   | 1.01 ± 0.05 n=10 | 0.99 ± 0.06 n=12 | n.s.                             |
|               | Ifng   | 1.31 ± 0.41 n=10 | 0.93 ± 0.10 n=11 | n.s.                             |
| Para-aortic LN† | Trav12 | 1.26 ± 0.22 n=10 | 5.56 ± 0.57 n=12 | p < .0001                        |
|               | Trbv31 | 1.04 ± 0.10 n=10 | 3.06 ± 0.30 n=12 | p = .0003                        |
|               | Tbx21  | 1.06 ± 0.12 n=10 | 0.96 ± 0.04 n=12 | n.s.                             |
|               | Gata3  | 1.02 ± 0.07 n=10 | 0.98 ± 0.06 n=12 | n.s.                             |
|               | Rorc   | 1.04 ± 0.09 n=10 | 1.31 ± 0.13 n=12 | n.s.                             |
|               | Foxp3  | 1.04 ± 0.11 n=10 | 0.88 ± 0.07 n=12 | n.s.                             |
|               | Il5    | 1.05 ± 0.11 n=10 | 1.69 ± 0.52 n=12 | n.s.                             |
|               | Il6    | 1.11 ± 0.21 n=10 | 0.71 ± 0.04 n=12 | p = .02                          |
|               | Il10   | 1.01 ± 0.05 n=10 | 0.99 ± 0.06 n=12 | n.s.                             |
|               | Ifng   | 1.09 ± 0.18 n=10 | 0.90 ± 0.06 n=12 | n.s.                             |
| Liver         | APOB   | 1.05 ± 0.12 n=10 | 1.10 ± 0.12 n=14 | n.s.                             |
|               | Col1a1 | 1.26 ± 0.27 n=10 | 1.03 ± 0.19 n=14 | n.s.                             |
|               | Col3a1 | 1.32 ± 0.35 n=10 | 1.38 ± 0.23 n=14 | n.s.                             |
|               | Tnf    | 1.03 ± 0.59 n=10 | 0.95 ± 0.08 n=14 | n.s.                             |
|               | Tgfb1  | 1.26 ± 0.86 n=10 | 1.21 ± 0.71 n=14 | n.s.                             |
|               | Ifng   | 1.77 ± 0.59 n=10 | 2.22 ± 0.45 n=14 | n.s.                             |

* Data calculated as relative expression using the 2^ΔΔCt formula, normalized to the house-keeping gene Hprt
†LN=lymph nodes
Table S5. Phenotype of \textit{HuBL} vs. \textit{BT3xHuBL} mice*

|                  | \textit{HuBL}          | \textit{BT3xHuBL}         | Significance (Student’s $t$-test) |
|------------------|------------------------|----------------------------|---------------------------------|
| **Body weight**  | 30.2 ± 0.5 n=16        | 24.6 ± 0.7 n=12           | $p = .003$                      |
| (g)              |                        |                            |                                 |
| **Blood WBC**†   | 12.1 ± 0.7 n=16        | 15.1 ± 1.1 n=12           | $p = .03$                       |
| ($10^9$/l)       |                        |                            |                                 |
| **Blood Lymphocytes** | 7.64 ± 0.55 n=16 | 7.62 ± 2.51 n=12         | n.s.‡                           |
| ($10^9$/l)       |                        |                            |                                 |
| **Blood Monocytes** | 0.51 ± 0.04 n=16     | 0.74 ± 0.07 n=12          | $p = .004$                      |
| ($10^9$/l)       |                        |                            |                                 |
| **Blood Granulocytes** | 3.91 ± 0.66 n=16 | 6.73 ± 0.90 n=12          | $p = .006$                      |
| ($10^9$/l)       |                        |                            |                                 |
| **Spleen weight**| 109 ± 5 n=11           | 175 ± 16 n=12             | $p = .002$                      |
| (mg)             |                        |                            |                                 |
| **Spleen Lymphocytes** | 141 ± 45 n=11        | 152 ± 38 n=12             | n.s.                            |
| ($10^6$)         |                        |                            |                                 |
| **Spleen Monocytes** | 3.64 ± 0.59 n=11     | 9.17 ± 1.09 n=12          | $p = .001$                      |
| ($10^6$)         |                        |                            |                                 |
| **Spleen Granulocytes** | 15.3 ± 1.6 n=11     | 48.0 ± 9.3 n=12           | $p = .0007$                     |
| ($10^6$)         |                        |                            |                                 |
| **Plasma ALT§**  | 23.6 ± 5.3 n=11        | 28.9 ± 7.1 n=16           | n.s.                            |
| (U/l)            |                        |                            |                                 |

* After a western diet period of 10 weeks  
† WBC=White blood cells  
‡ n.s.=not significant  
§ ALT=Alanine transaminase
Supplemental Methods

Lipoprotein preparations

LDL (density 1.019 – 1.063 g/ml) was isolated by ultracentrifugation from pooled plasma of healthy donors and dialyzed extensively against PBS. One mM EDTA was added to prevent oxidation. Using the same procedure, mouse LDL was prepared from plasma of Ldlr−/− mice. Highly oxidized LDL was obtained by incubating LDL (1 mg/ml protein content) with 20 μM CuSO₄ for 18 hours at 37°C. Soluble ApoB100 was isolated as previously described. Briefly, protein was precipitated from LDL, resuspended in sodium dodecyl sulfate, filtered on a PD-10 column (GE Healthcare), and further purified on a Superdex-200 size exclusion column (0.5 ml/min, in Tris-HCl buffer, pH 7.4). FITC-LDL and FITC-oxLDL was prepared as previously described.

Macrophage oxLDL uptake assay

RAW264.7 mouse macrophage-like cells (ATCC) were used to evaluate uptake of oxLDL. Two-hundred thousand cells per well were seeded into 48-well cell culture plates (Corning) in 1% FBS RPMI 1640 medium (Thermo Fisher Scientific) with 25 µg/ml FITC-labeled oxLDL. Plasma was dialyzed against PBS and added at a dilution of 1:100. Cellular FITC signals were recorded with an IncuCyte ZOOM live-cell imaging and analysis platform (Essen Bioscience). After 24 hours incubation at 37°C in a humid 5% CO₂ atmosphere, four images per well from six replicates were taken using a 20x objective and subsequently analyzed using the IncuCyte Basic Software. The cells were then washed, fixed in PBS-buffered 4% formaldehyde solution, and stained with hematoxylin and Oil Red O. Photomicrographs were acquired using a 40x objective and a Leica DMIL inverted microscope.

Lipoprotein clearance studies

For lipoprotein clearance studies, 100 μg FITC-LDL was mixed with 70% mouse plasma from HuBL mice injected with either B6 or BT3 CD4+ T cells, and injected into the tail vein of male HuBL mice. Blood samples were collected in EDTA-coated tubes 1, 5, 15, 30, and 60 minutes after the injection. IgG purification was performed using Protein G resin (GE Healthcare). After dialysis against PBS, the protein concentration was determined with a Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific). Two-hundred μg of the isolated IgGs were infused into 10-week-old male HuBL recipients and blood samples were collected before and at 15, 60, and 360 minutes after the infusion.

RNA isolation, cDNA synthesis, and real-time PCR

RNA was isolated from aorta, para-aortic lymph nodes, liver, and thymus using RNeasy kit (Qiagen). Total RNA quality was analyzed on a BioAnalyzer instrument (Agilent Technologies) and was quantified by 260 nm absorbance measurement using a Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific). Reverse transcription was performed with high-capacity cDNA reverse transcription kit and amplification was performed by real-time-PCR using TaqMan Universal Master Mix and pre-manufactured primers and probes for the genes of interest and for hypoxanthine guanine phosphoribosyl transferase (Hprt, assay-on-demand), in an ABI 7900HT Fast Real-Time PCR System (all Applied Biosystems). For Trav12 and Trbv31 mRNA, specific primers and probe were used as previously described. Data were analyzed using relative expression with the formula 2−ΔΔCt, where ΔΔCt = ΔCt (sample) − ΔCt (mean Ct values of the controls), and ΔCt is the Ct of the housekeeping gene (Hprt) subtracted from the Ct of the target gene.

Immunohistochemistry and immunofluorescent analysis

Primary antibodies to CD4 (clone H129.19), CD8 (clone 53-6.7), VCAM-1 (clone 429; all from BD Biosciences), α-SM-actin (ab5694, Abcam), and CD68 (clone FA-11, AbD Serotec) were applied to acetone-fixed cryosections, followed by biotinylated rabbit anti-rat IgG. The primary antibody for IAβ (clone KH74, BD Biosciences) was a biotin-conjugated IgG. Staining was visualized with Vectastain ABC kit and diaminobenzidine (Vector). Quantification of immunohistochemical staining was documented as the ratio of thresholded chromogen.
area using QWin Standard Y 2.8 computerized analysis (Leica) or calculated as the number of stained cells divided by the intimal lesion area. For fluorescent staining of spleen, liver, kidney, and duodenum sections, antibodies against B220 (clone RA3-6B2), F4/80 (clone BM8; both from BioLegend), IgG1 (1070, Southern Biotech), IgG2c (clone 5.7), CD138 (clone 281-1; both from BD Biosciences), C3 (A0062, Dako), and ApoB (20-AG40, Fitzgerald) were used. Horse anti-goat IgG (Dylight 488, Vector) was used as secondary antibody for ApoB staining.

Plasma analysis

Plasma was separated through centrifugation of whole blood, 1500g for 15 minutes. Plasma creatinine levels were analyzed using a colorimetric assay (Cayman Chemical Company) relying on the Jaffe’ reaction. Plasma alanine transaminase activity was measured using a colorimetric assay kit (Abcam). Plasma ApoB levels were measured with a human ApoB ELISA development kit (Mabtech). For FITC-LDL detection, plasma was diluted 1:25 in PBS and FITC fluorescence was analyzed using a Wallac 1420 Victor2 reader (Perkin Elmer).

Cholesterol extraction from liver and feces

Feces was collected during eight hours the day before sacrifice of the mice. Upon sacrifice, a piece of the liver was snap-frozen. One-hundred mg tissue was homogenized in methanol, and lipids were extracted by chloroform separation. After drying the extracts, they were redissolved and cholesterol content were measured with a colorimetric kit (Randox).

Proliferation assay

Splenocytes were isolated by meshing spleens from individual mice through a 100 µm cell strainer followed by osmotic lysis of red blood cells (EL buffer; Qiagen). Five-hundred thousand splenocytes were incubated in 96-well plates with 200 µl serum-free RPMI 1640 medium containing ITS Premix (Corning), 0.1% bovine serum albumin, nonessential amino acids, L-glutamine, 1 mM sodium pyruvate, and 50 µM β-Mercaptoethanol for 60 hours at 37°C in a humid 5% CO₂ atmosphere. Triplicates were used for all samples. The splenocytes were stimulated with 10 µg/ml human LDL, 10 µg/ml mouse LDL, 0.6 µg/ml Concanavalin A (Con A), or 1 µg/ml rat-anti mouse CD3 antibody (clone C363.29B, Southern Biotech) together with 2 µg/ml rat-anti mouse CD28 antibody (clone 37.51, eBioscience). One µCi ³H-thymidine (Perkin Elmer) was added after 48 hours and DNA replication was measured in a scintillation counter (Wallac). Results are expressed as counts per minute (CPM) or stimulation index, calculated as CPM of the stimulated cells subtracted with the CPM of unstimulated cells from the same animal, and then divided with the CPM of unstimulated cells. IFN-γ levels in supernatants were measured by a commercial ELISA kit (Mabtech).

Supplemental References

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3. Klingenberg R, Gerdes N, Badeau RM, Gistera A, Strothoff D, Ketelhuth DF, Lundberg AM, Rudling M, Nilsson SK, Olivecrona G, Zoller S, Lohmann C, Luschert TF, Jauhiainen M, Sparwasser T and Hansson GK. Depletion of FOXP3+ regulatory T cells promotes hypercholesterolemia and atherosclerosis. J Clin Invest. 2013;123:1323-34.
List of antibodies used for flow cytometry analysis.

| Target          | Clone     | Company      | Conjugation     |
|-----------------|-----------|--------------|-----------------|
| B220            | RA3-6B2   | BioLegend    | APC-Cy7         |
| CD3ε            | 145-2C11  | BioLegend    | PerCP           |
| CD4             | GK1.5     | BD Biosciences | APCH7          |
| CD8α            | 53-6.7    | BD Biosciences | PerCP          |
| CD19            | ID3       | BD Biosciences | PE            |
| CD25            | PC61.5    | eBioscience  | PE-Cy7         |
| CD28            | E18       | BioLegend    | FITC           |
| CD44            | IM7       | BioLegend    | PerCP           |
| CD62L           | MEL-14    | BioLegend    | APC-Cy7         |
| CD69            | H1.2F3    | BD Biosciences | PerCP-Cy5.5   |
| CD95            | Jo2       | BD Biosciences | PE-Cy7         |
| CD138           | 281-2     | BD Biosciences | APC           |
| CXCR5           | 2G8       | BD Biosciences | Biotin         |
| Foxp3           | FJK-16s   | eBioscience  | PE              |
| GL7             | GL7       | eBioscience  | APC             |
| IgD             | 11-26c.2a | BioLegend    | PerCP           |
| PD1 (CD279)     | J43       | BD Biosciences | PE            |
| Tbet            | 4B10      | BioLegend    | Brilliant Violet 421 |
| TRBV31 (Vβ14 TCR) | 14-2   | BD Biosciences | FITC, biotin  |