SUPPLEMENTAL METHODS

BM transplantation

BM cells were collected from the both femurs and tibiae of donor C57BL/6-Ly5.1 mice (kindly gifted by Dr. Yoshimura) (Young, 6–8 weeks old; Old, > 20 weeks old) and 10^7 cells were intravenously injected into lethally irradiated (10 Gy X-rays) male WT recipients (C57BL/6J-Ly5.2 mice) (Young, 6–8 weeks old; Old, > 20 weeks old). At 28 days post-transplantation, BM cells and PBL were collected from all chimeras.

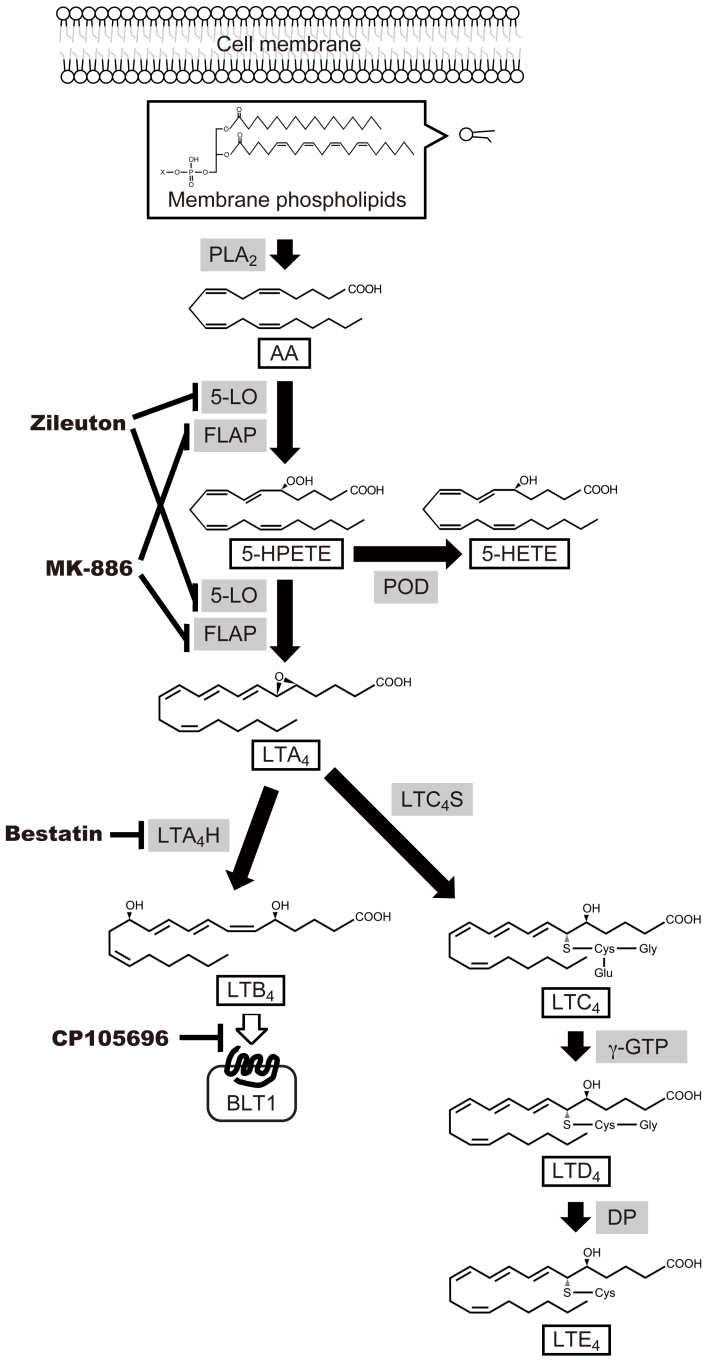
Flow cytometry analysis

BM cells and PBL were incubated with mouse Fc blocker, followed by staining with anti-CD45-FITC (2.5 μg/ml; clone 30-F11), anti-CD45.2-PE (2.5 μg/ml; clone 104) (Thermo Fisher Scientific/eBioscience), and anti-CD45.1-APC (2.5 μg/ml; clone A20) (Thermo Fisher Scientific/eBioscience). RAW274.7 cells were pretreated with 5 μM Pyridone 6 (a JAK1 inhibitor) or 2 μM Cucurbitacin I (a STAT3 inhibitor) (purchased from EMD Millipore, Billerica, MA), or DMSO (vehicle, 0.05%) for 1 h, were then polarized by exposure to IL-4, IL-10, IL-13, and TGF-β1 (M2) or IFN-γ and LPS (M1) for 24 h. Cells were stained with anti-F4/80-FITC (5 μg/ml), anti-CD11b-APC (1.25 μg/ml), and biotin-labeled anti-mouse BLT1 (1.25 μg/ml) or mouse IgG_1 (1.25 μg/ml) Abs. After washing with PBS/EDTA, cells were stained with Streptavidin-PE (0.6 μg/ml). Cells were washed and then analyzed in a flow cytometer (FACSCalibur or FACSVerse). For all experiments, dead cells were excluded after staining with 7AAD.

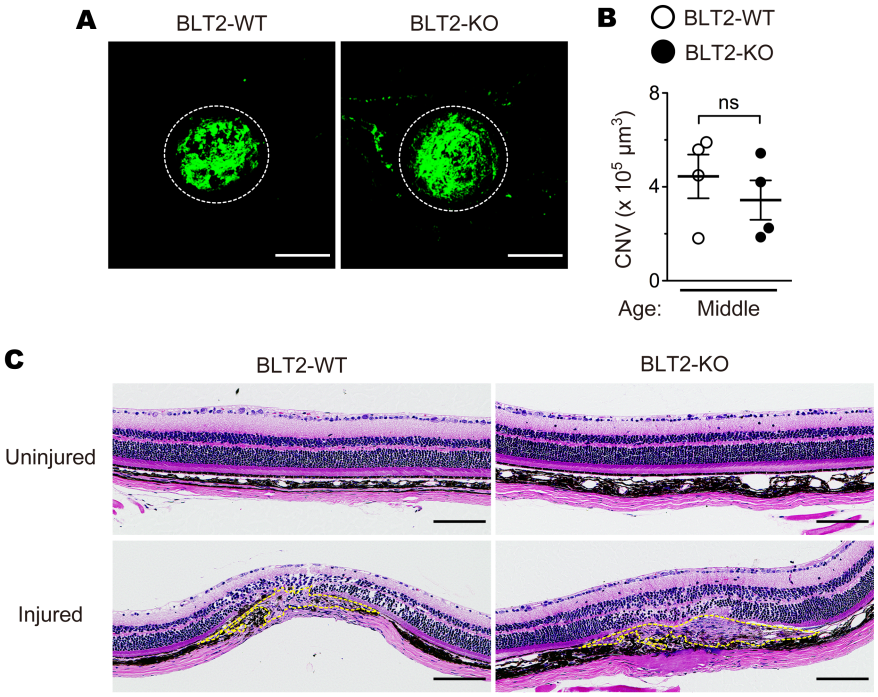
Quantitative RT-PCR

BM cells from WT mice were differentiated into BMDMs by treatment with 50 ng/ml

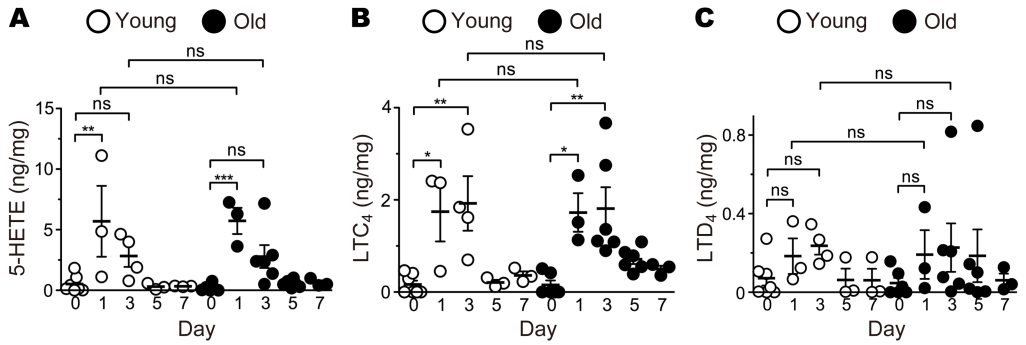
M-CSF for 5 days. BMDMs were cultured without M-CSF for 1 day, were then polarized by 2 ng/ml mouse IFN-γ and 0.1 μg/ml LPS (M1) for 24 h or 50 ng/ml IL-4, IL-10, and IL-13 (from mouse) and 4 ng/ml human TGF-β1 (M2) for 48 h. M0 macrophages were generated by culture in medium without cytokines. cDNA from M0-, M1-, and M2-BMDMs was synthesized from total RNA using QuantiTect Reverse Transcription kit. Target genes were amplified using a real-time PCR System, DNA polymerase, SYBR Green I Dye and specific primers. Gene expression was normalized to 18S rRNA (*Rn18s*) using the ΔΔCT method. The sequences of the primers are as follows: *Ltb4r1*: Forward, 5'-ctcggaggtgtccagcac-3' and Reverse, 5'-gacaggcaggtgtgtccttc-3'; *Rn18s*: Forward, 5'-gcaattattccccatgaacg-3' and Reverse, 5'-gggacttaatcaacgcaagc-3'; *Mrc1*: Forward, 5'-ccacagcattgaggagtttg-3', and Reverse, 5'-acagctcatcatttggctca-3'; *Mgl2*: Forward, 5'-ggagtctccaaagtttgctctaa-3', and Reverse, 5'-aggtgggtccaagagaggat-3'; *Pdcd1lg2*: Forward, 5'-tgtgctgccttttctgtgtc-3', and Reverse, 5'-gcagcatggtctgtgtcaat-3'; *Arg1*: Forward, 5'-cctgaaggaactgaaaggaaag-3', and Reverse, 5'-ttggcagatatgcagggagt-3'; *Retnla*: Forward, 5'-ccctccactgtaacgaagactc-3', and Reverse, 5'-cacacccagtagcagtcatcc-3'; *Chil3*: Forward, 5'-gaacactgagctaaaaactctcctg-3', and Reverse, 5'-gagaccatggcactgaacg-3'; *Nos2*: Forward, 5'-gggctgtcacggagatca-3', and Reverse, 5'-ccatgatggtcacattctgc-3'.



Supplemental Figure 1. The biosynthetic pathway for the leukotrienes.
LTB_4 is generated from arachidonic acid by 5-LO, FLAP, and LTA_4H. BLT1 is a high affinity receptor for LTB_4. Zileuton, 5-LO inhibitor; MK-886, FLAP inhibitor; Bestatin, LTA_4H inhibitor; CP105696, BLT1 antagonist. AA, arachidonic acid; 5-HPETE, 5-hydroperoxyeicosatetraenoic acid; 5-HETE, 5-hydroxyeicosatetraenoic acid; LTA_4, leukotriene A_4; LTB_4, leukotriene B_4; LTC_4, leukotriene C_4; LTD_4, leukotriene D_4; LTE_4, leukotriene E_4; Cys, cysteine; Glu, glutamate; Gly, glycine; PLA_2, phospholipase A_2; FLAP, 5-lipoxygenase-activating protein; 5-LO, 5-lipoxygenase; POD, peroxidase; LTA_4H, LTA_4 hydrolase; LTC_4S, LTC_4 synthase; γ-GTP, γ-glutamyltranspeptidase; DP, dipeptidase.

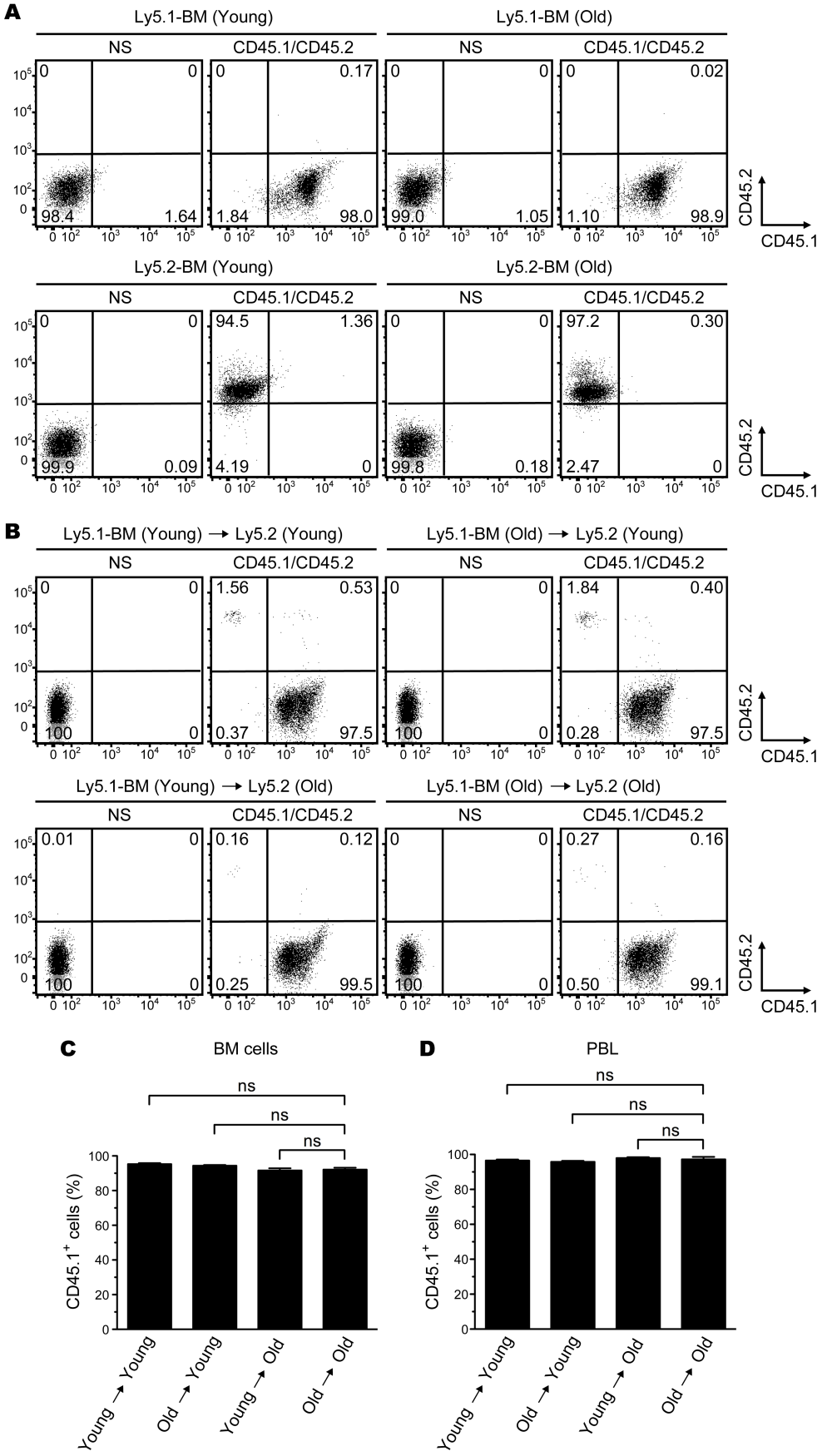


Supplemental Figure 2. BLT2 is not involved in the AMD pathogenesis. CNV images (A) and volume (B) in the laser-injured RPE-choroid from aged BLT2-WT (open circles) and BLT2-KO (filled circles) mice (> 20 weeks old). *n*=4 mice per group. (C) H&E staining of the uninjured and laser-injured retinas from aged BLT2-WT and BLT2-KO mice (> 20 weeks old). Yellow dotted lines denote the lesion areas. Bar=100 µm (A, C). (B) ns, not significant (Student's *t* test). Results are representative of at least two independent experiments.



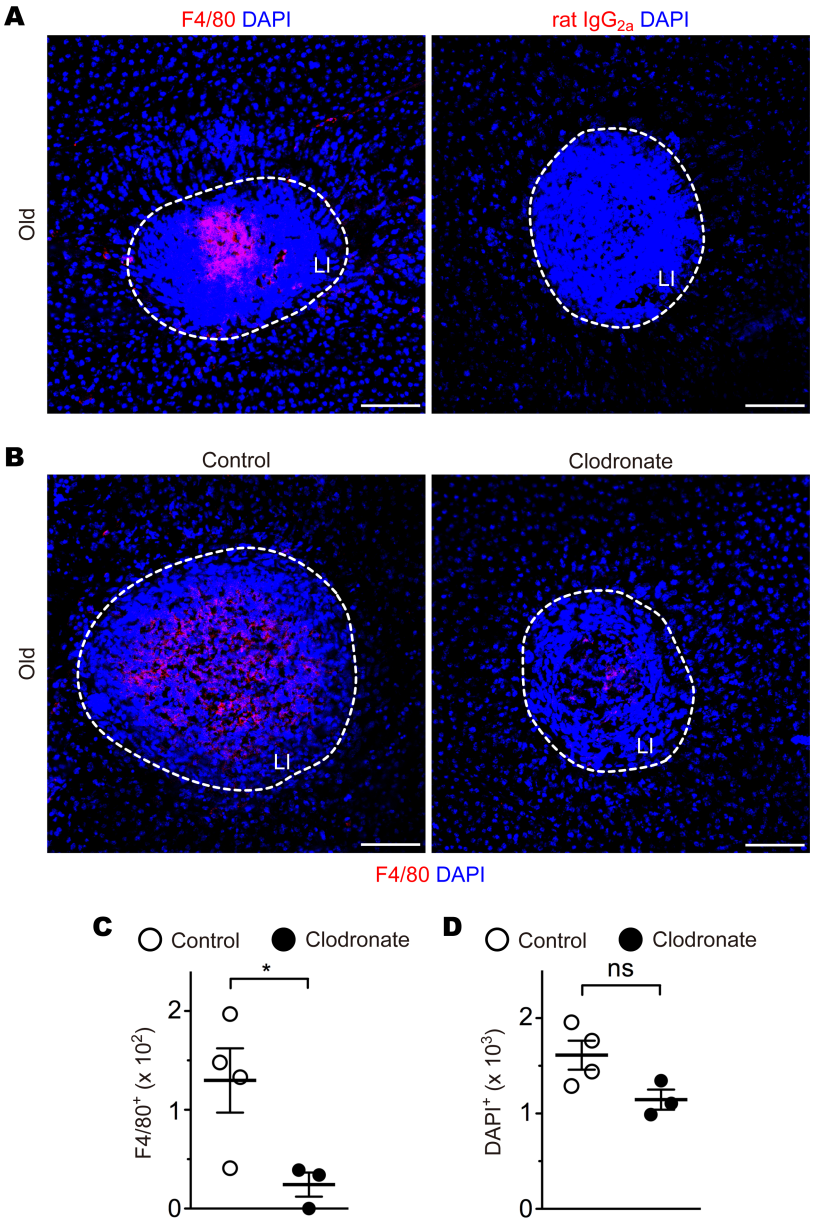
Supplemental Figure 3. Levels of 5-HETE, LTC_4, and LTD_4 in the laser-injured eyes.

Time course of 5-HETE (A), LTC_4 (B), and LTD_4 (C) accumulation in laser-injured eyes of young (white bars) and old (black bars) WT mice (Young, 8 weeks old; Old, > 20 weeks old). n=3–7 per group. (A-C) *$P < 0.05$; **$P < 0.01$; ***$P < 0.005$; ns, not significant (1-way ANOVA with the Bonferroni's *post hoc* test). Results are representative of at least two independent experiments.



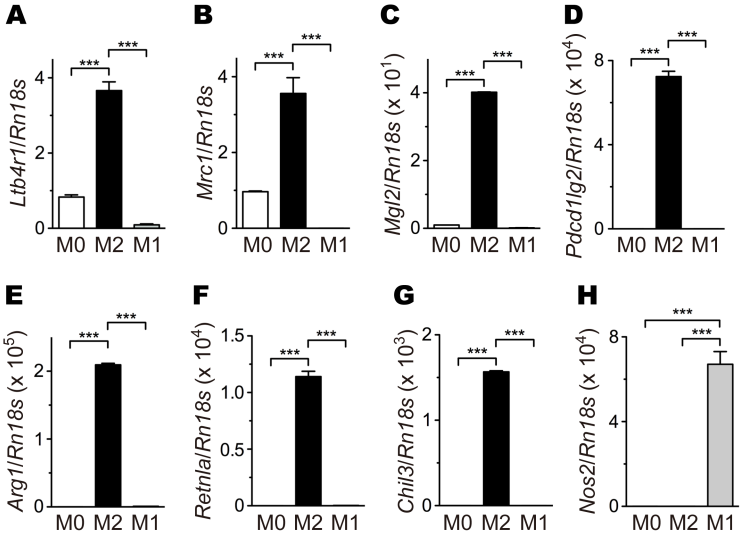
67 Supplemental Figure 4. Generation of BM chimeras using aged donor and

recipient.
(A) FACS analysis of a congenic marker in the BM cells from Ly5.1 (Ly5.1-BM) and Ly5.2 (Ly5.2-BM) mice (Young, 8 weeks old; Old, > 20 weeks old). Ly5.1 and Ly5.2 mice have $CD45.1^+$ and $CD45.2^+$ leukocytes, respectively. (B) FACS analysis of a congenic marker in the PBL from BM chimeras; Ly5.1-BM cells (Young) into Ly5.2 mice (Young), Ly5.1-BM cells (Old) into Ly5.2 mice (Young), L5.1-BM cells (Young) into Ly5.2 mice (Old), Ly5.1-BM cells (Old) into Ly5.2 mice (Old). (C) Replacement rate of $CD45^+$ leukocytes in the BM cells and PBL from BM chimeras. n=3–7 per group. These data were obtained using BM transplantation as described in Supplemental Methods. ns, not significant (1-way ANOVA with the Bonferroni's *post hoc* test). Young into Young, Ly5.1-BM cells (Young) into Ly5.2 mice (Young); Old into Young, Ly5.1-BM cells (Old) into Ly5.2 mice (Young); Young into Old, L5.1-BM cells (Young) into Ly5.2 mice (Old); Old into Old, Ly5.1-BM cells (Old) into Ly5.2 mice (Old).

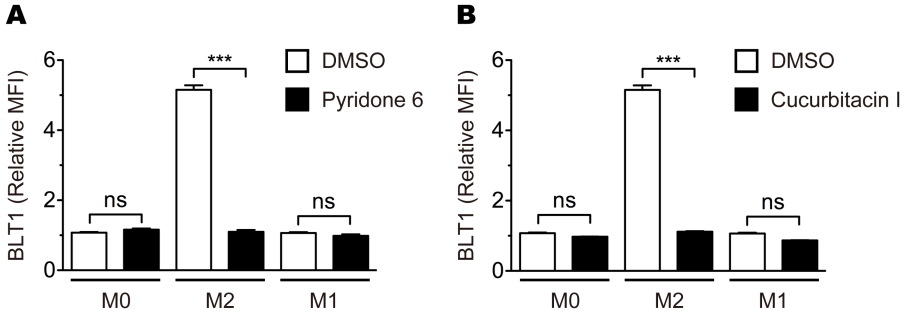


Supplemental Figure 5. Depletion of macrophages in the laser-injured RPE-choroid using clodronate liposome.

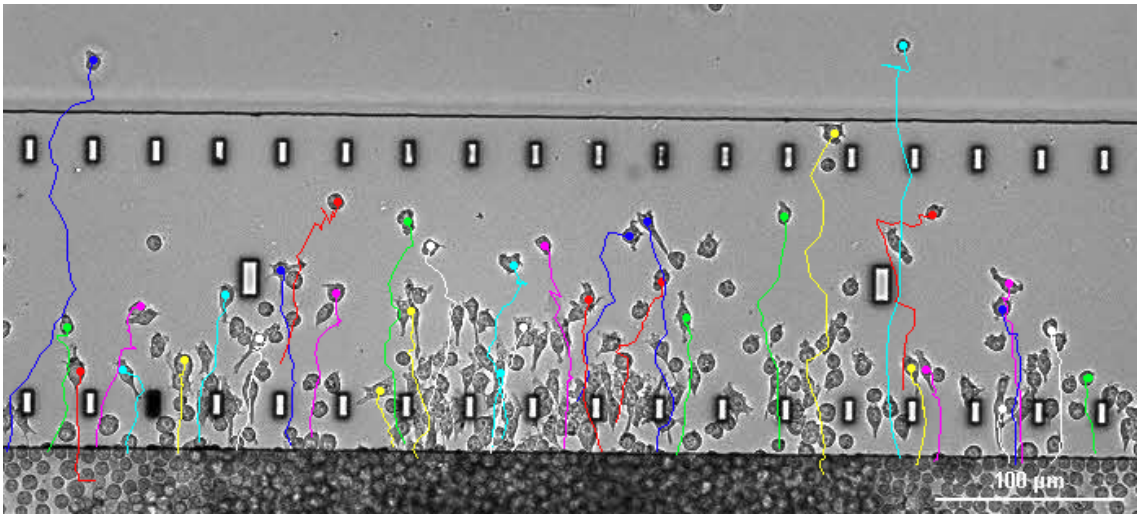
(A, B) Immunofluorescence staining of uninjected (A) and clodronate or control liposome-injected (B) RPE-choroid from aged WT mice (> 20 weeks old) with anti-F4/80 mAb or rat IgG_{2a} as an isotype control (red). Eyes were collected on Day 5 post-laser injury. Nuclei were visualized with DAPI (blue). LI (white dotted lines) denotes the location of laser injury. Control, control liposome; Clodronate, clodronate liposome. (C, D) The number of $F4/80^+$ (C) and $DAPI^+$ (D) cells was counted as described above. n=3-4 per group. Bar=100μm (A, B). (C, D) *P < 0.05; ns, not significant (Student's t test).



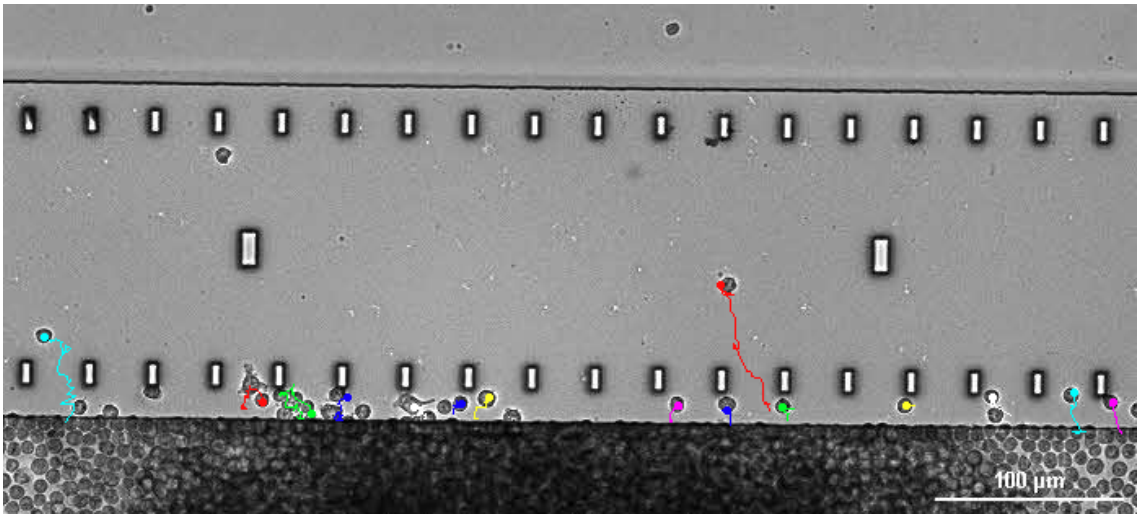
92 **Supplemental Figure 6. mRNA expression of M1 and M2 macrophage markers.**
93 Quantitative RT-PCR analysis of mRNA for various M1 and M2 macrophage markers
94 in the M0-, M2, or M1-BMDMs from WT mice as described in Supplemental Methods.
95 n=3 per group. (A-H) ***$P < 0.005$ (1-way ANOVA with Bonferroni's *post hoc* test).



Supplemental Figure 7. Down-regulation of BLT1 expression in M2-polarized RAW264.7 cells by inhibition of the JAK1-STAT3 signaling pathway.
FACS analysis of BLT1 expression in M2-RAW264.7 cells after treatment of 5 μM Pyridone 6 (a JAK1 inhibitor) or 2 μM Cucurbitacin I (a STAT3 inhibitor) (black bars), or vehicle (white bars). The Y-axis shows the MFI relative to that of an isotype control (mouse IgG_1). MFI, mean fluorescence intensity. n=3–9 per group. (A, B) ***P < 0.005; ns, not significant (1-way ANOVA with the Bonferroni's *post hoc* test).



Supplemental movie 1. Chemotaxis of M2-polarized RAW264.7 cells to LTB_4. *In vitro* chemotaxis of M2-RAW264.7 cells toward 100 nM LTB_4 was analyzed using TAXIScan-FL device. Bar=100 µm.



Supplemental movie 2. Chemotaxis of M2-polarized RAW264.7 cells to vehicle control.

In vitro chemotaxis of M2-RAW264.7 cells toward ethanol was analyzed using TAXIScan-FL device. Bar=100 µm.