SUPPLEMENTARY DATA

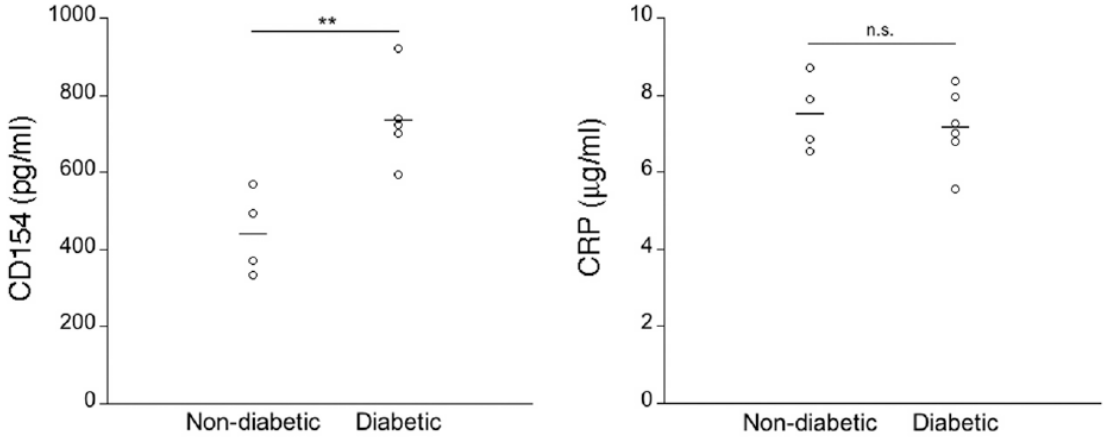
Antibodies

Sections were incubated with biotinylated anti-CD40 mAb (3/23, BioLegend) followed by incubation with anti-vimentin (RV202, Novus Biologicals), anti-CRALBP (15356-1-AP, Proteintech Group), anti-GFAP (GA5, EMD Millipore), anti-Iba-1 Ab (019-19741, Wako Chemicals), anti-mouse Brn-3a (sc-8429, Santa Cruz Biotechnology) or with Tomato Lectin DyLight 488 (Vector Laboratories). After citrate buffer treatment, sections were incubated with anti-ICAM-1 (KAT-1, eBiosciences), anti-TNF-α (52B83, Abcam), anti-CCL2 Ab (2D8, Novus Biologicals), anti-$P2X_7$ (AP09521PU-N, Acris) Abs plus either anti-Iba1, anti-vimentin, anti-CRALBP Abs or Tomato Lectin DyLight 488. Secondary Abs were from Jackson ImmunoResearch Laboratories. Staining specificity was confirmed by omitting primary Abs and by staining with primary Ab alone. Antibodies for flow cytometry were: anti-CD3 (17A2), anti-CD11b (M1/70), anti-CD31 (390), anti-CD40 (HM40-3), anti-CD49b (DX5), anti-B220 (RA3-6B2), anti-F4/80 (BM8), anti-Gr-1 (RB6-8C5), anti-Thy-1 (HIS51) (all from eBioscience, San Diego, CA); anti-vimentin (V9), anti-GFAP (N-18, both from Santa Cruz) and anti-rhodopsin (1D4).

$P2X_7$ receptor-dependent Ca^{2+} influx

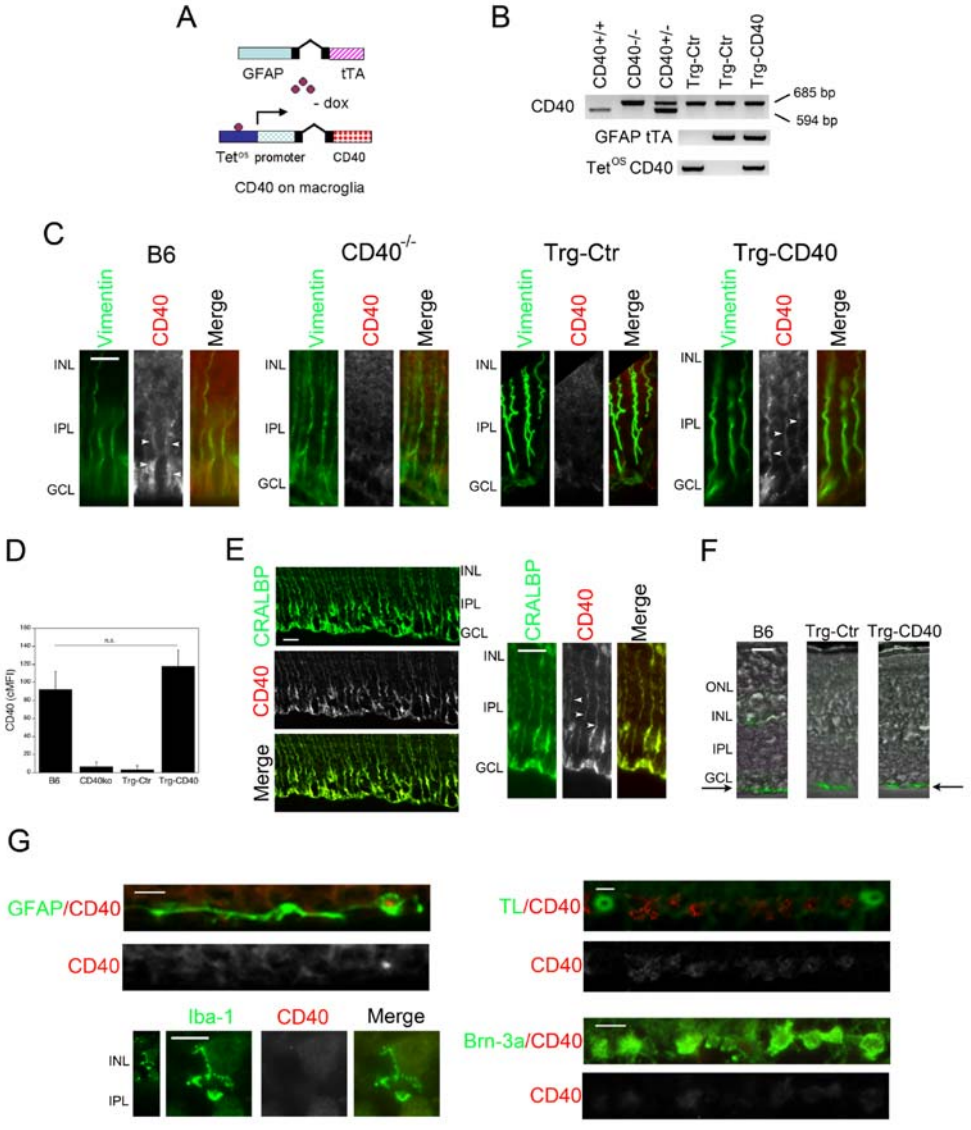
Müller cells or HEK293 cells (HEK-wt or HEK-hP2X7) were loaded with fluo-4 Ca^{2+} sensor dye and assayed for ATP-induced changes in cytosolic Ca^{2+}. Wells were supplemented with 10 μM AZ10606120 ($P2X_7$ antagonist) before stimulation with ATP. Baseline fluorescence was measured. The fluo-4-loaded cells were then stimulated with ATP. Fluorescence was measured prior to addition of 1% Triton X-100 to release the fluo-4 dye for determination of maximum Ca^{2+}-dependent fluorescence. Wells were then supplemented with 15 mM EGTA/50 mM Tris to determine Ca^{2+}-independent fluorescence.

Supplementary Figure 1. Serum concentrations of CD154 are increased in diabetic mice. At 2 m of diabetes, sera from diabetic B6 mice as well as from non-diabetic control animals were collected and used to measure concentration of soluble CD154 and CRP by ELISA. ** $P < 0.01$ by Student's *t* test.



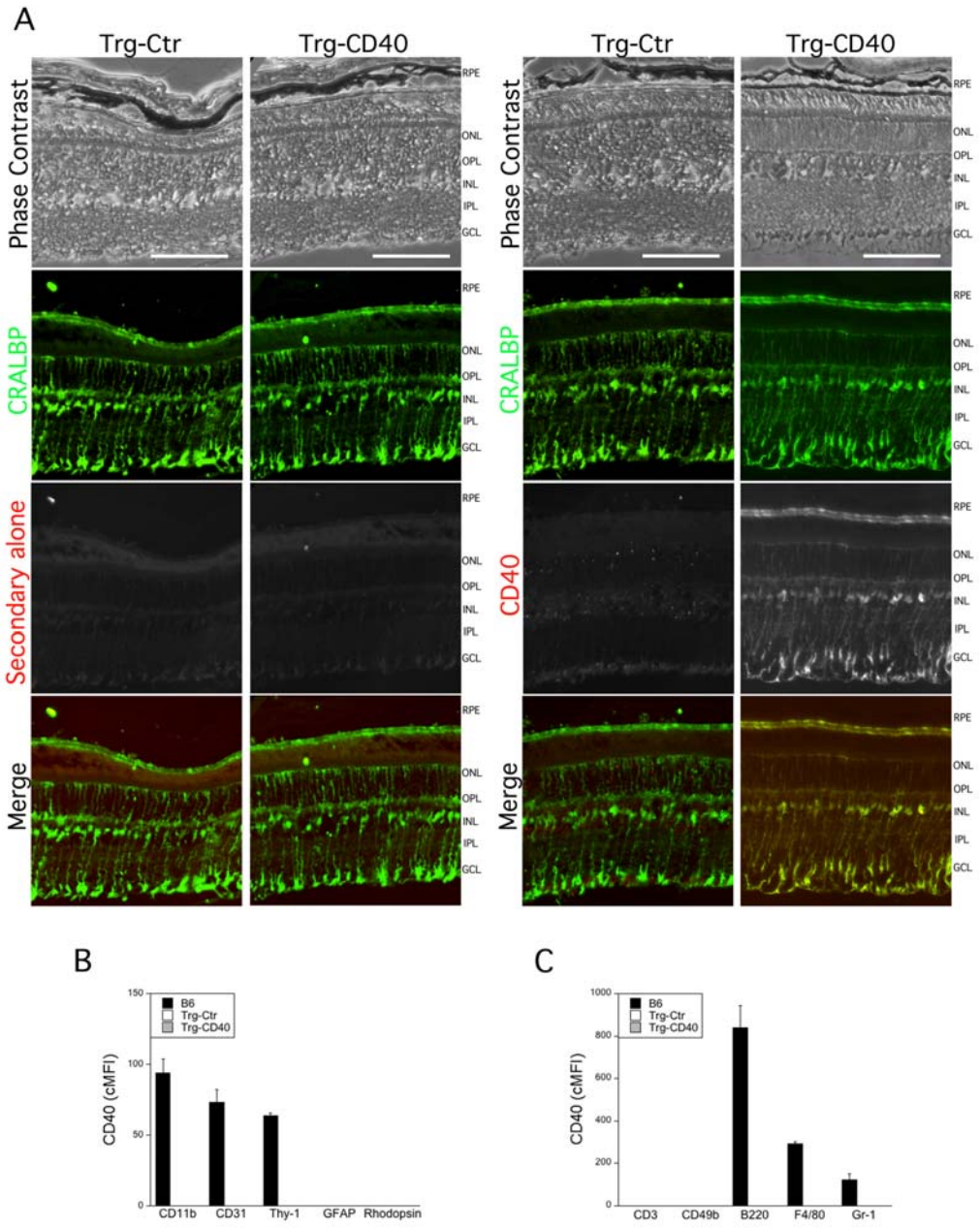
SUPPLEMENTARY DATA

Supplementary Figure 2. Double transgenic (Trg-CD40) mice express CD40 in retinal Müller cells. *A,* Binary tetracycline (Tet) repressible system. *B,* PCR of genomic DNA was performed using primers for CD40 (WT or mutant that results in results in lack of functional CD40), tTA and Tet^{OS} CD40. The PCR product for WT CD40 is 594 bp while the PCR product for mutant CD40 is 685 bp. *C,* CD40 expression in Müller cells ($vimentin^+$) from B6, $CD40^{-/-}$, single transgenic (Trg-Ctr) or double transgenic (Trg-CD40) mice. *D,* Retinal cells were incubated with anti-CD40 mAb plus anti-vimentin Ab. Corrected mean fluorescence intensity (cMFI) for CD40 on gated $vimentin^+$ cells. Data represents mean ± SEM of 3 mice. n.s. = non significant. *E,* CD40 expression in $CRALBP^+$ (specific Müller cell marker) cells from Trg-CD40 mice. Arrowheads show CD40 expression in $vimentin^+$ or $CRALBP^+$ cells. *F,* GFAP expression in B6, Trg-Ctr and Trg-CD40 mice. GFAP expression was noted in astrocytic processes present in the ganglion cell layer (arrow). *G,* CD40 expression in astrocytes ($GFAP^+$), endothelial cells ($Tomato\ lectin^+$), microglia/macrophage ($Iba1^+$) and ganglion cells ($Brn\text{-}3a^+$) from Trg-CD40 mice. GCL = Ganglion cell layer; IPL = Inner plexiform layer; INL = Inner nuclear layer. ONL = Outer nuclear layer. Scale bar, 10 μm. 8-12 sections per mouse; 4 mice/group.



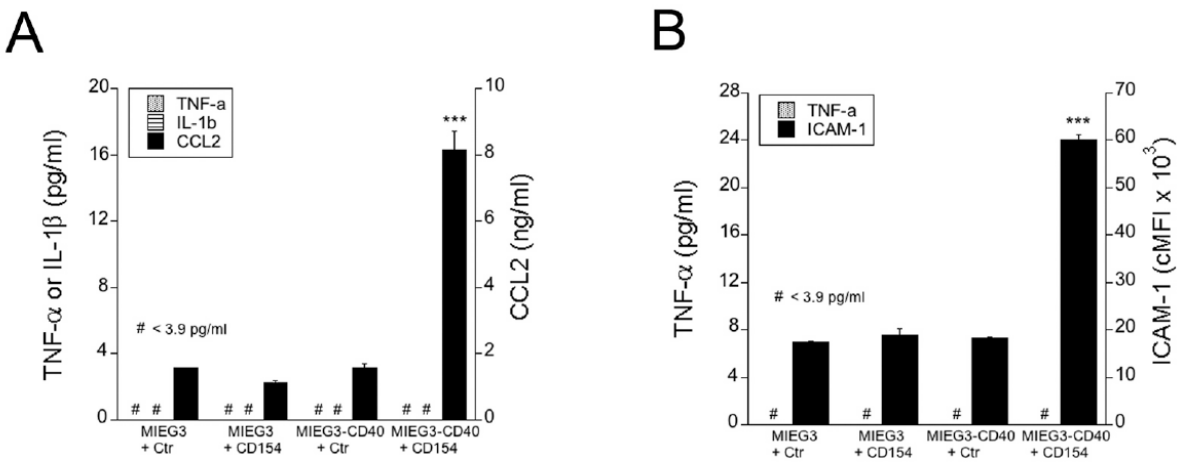
SUPPLEMENTARY DATA

Supplementary Figure 3. Double transgenic (Trg-CD40) mice express CD40 in retinal Müller cells. *A,* CD40 expression retinas from Trg-Ctr and Trg-CD40 mice. GCL = Ganglion cell layer; IPL = Inner plexiform layer; INL = Inner nuclear layer; OPL = Outer plexiform layer; ONL = Outer nuclear layer; RPE = retinal pigment epithelial cells. Scale bar, 100 μm. 4 mice/group. *B*, Retinal cells were incubated with anti-CD40 mAb plus antibodies against CD11b (microglia/macrophage), CD31 (endothelial cells), Thy-1 (ganglion neurons), GFAP (astrocytes) and rhodopsin (photoreceptors). cMFI for CD40 on gated $CD11b^{+}$, $CD31^{+}$, $Thy\text{-}1^{+}$, $GFAP^{+}$ or $rhodopsin^{+}$ cells calculated as described (22). *C*, CD40 expression on leukocyte subsets that express CD3 (T cell marker), CD49b (Natural Killer cell marker), B220 (B cell marker), F4/80 (macrophage marker) or Gr-1 (neutrophil marker) isolated from the spleen. The phenotype of Trg-Ctr mice was identical as that of $CD40^{-/-}$ mice (not shown). Data represents mean ± SEM of 4 mice.



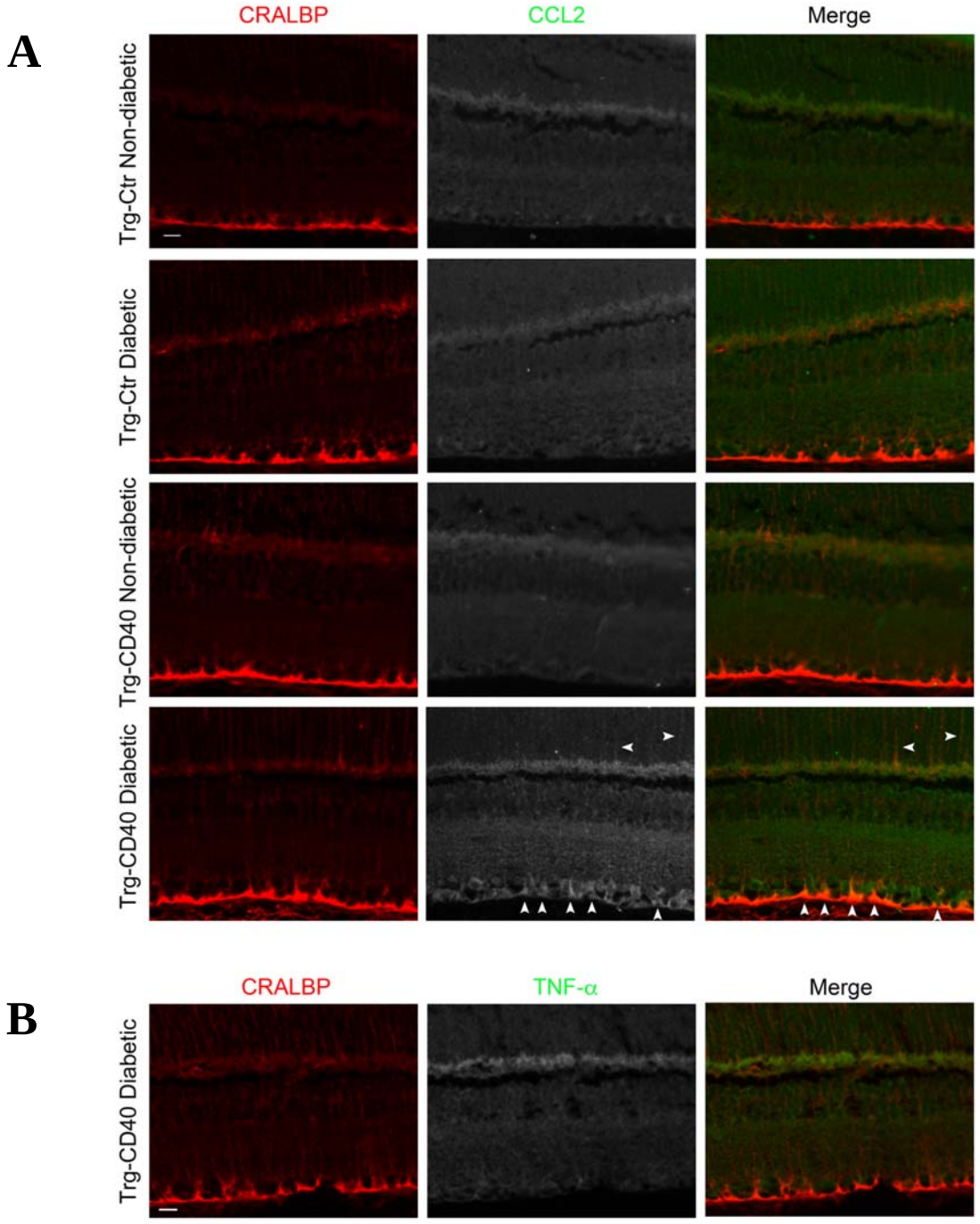
SUPPLEMENTARY DATA

Supplementary Figure 4. **Retinal Müller cells do not secrete TNF-α or IL-1β in response to CD40 ligation**. *A,* Human Müller cells transduced with CD40-encoding retroviral vector (MIEG3-CD40) were incubated with or without CD154 for 24 h. TNF-α, IL-1β and CCL2 were measured in supernatants using ELISA. *B,* Rat Müller cells (rMC-1) transduced with a retroviral vector that encodes human-mouse CD40 chimera were incubated with or without human CD154 for 24 h. TNF-α was measured by ELISA and ICAM-1 was assessed by flow cytometry. Results are presented as mean + SEM (n = 3). *** $P < 0.001$ by Student's *t* test.



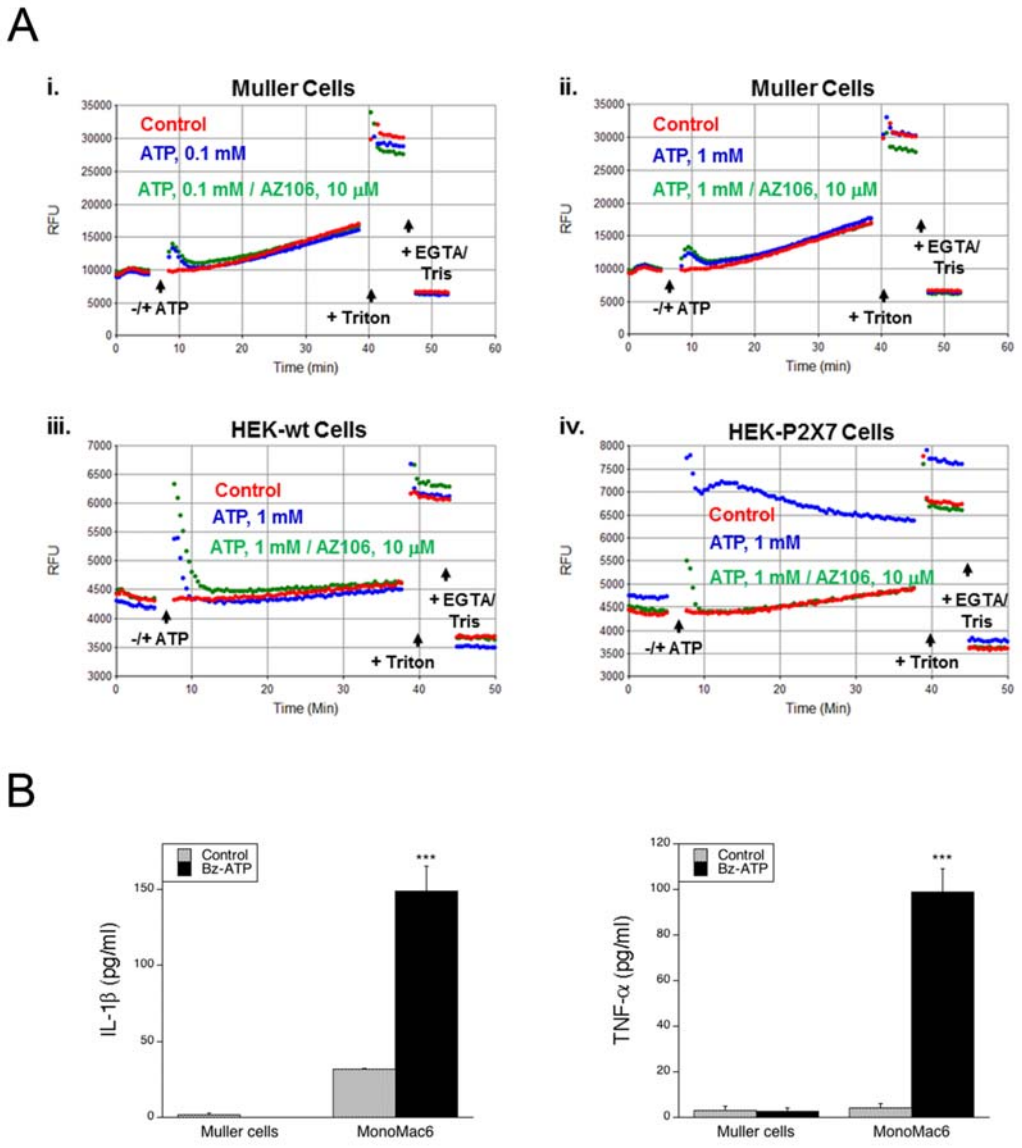
SUPPLEMENTARY DATA

Supplementary Figure 5. Diabetic transgenic mice that express CD40 in retinal Müller cells upregulate CCL2 but not TNF-α in Müller cells. *A*, Retinal sections from diabetic Trg-Ctr and Trg-CD40 mice at 2 m of diabetes and from non-diabetic controls were incubated with anti-CCL2 plus anti-CRALBP Ab. Co-localization of CCL2 and CRALBP is noted in diabetic Trg-CD40 mice (arrowheads). *B*, Retinal sections from diabetic Trg-CD40 mice at 2 m of diabetes were incubated with anti-TNF-α mAb plus anti-CRALBP Ab. Scale bar, 10 μm. 4 mice/group.



SUPPLEMENTARY DATA

Supplementary Figure 6. **Human retinal Müller cells do not appear to express functional $P2X_7$ receptor while monocytic cells secrete IL-1β and TNF-α in response to a $P2X_7$ receptor ligand**. *A*, Müller cells (panels i and ii), control HEK293 cells (HEK-wt; panel iii), or HEK293 transfected with human $P2X_7$ receptor cDNA (panel iv) were loaded with fluo-4 Ca^{2+} indicator dye, pretreated with (green) or without (red, blue) 10 μM AZ10606210 for 15 min and then assayed for ATP-induced increases in cytosolic [Ca^{2+}]. In panel a-i, the Müller cells were stimulated with 0.1 mM ATP while in panels a-ii, a-iii, and a-iv, the Müller cells, HEK-wt, or HEK-P2X7 cells were stimulated with 1 mM ATP. After the indicated times, the cells were permeabilized with 1% triton-X100 to release intracellular fluo-4 into the extracellular medium followed by addition of EGTA and Tris to remove Ca^{2+} bound to fluo-4. *B,* Müller cells and MonoMac6 cells were incubated with or without Bz-ATP (300 μM). IL-1β and TNF-α were measured by ELISA. Results are presented as mean + SEM (n = 3). ****P* < 0.001 by Student's *t* test.



SUPPLEMENTARY DATA

Supplementary Table 1. Average weight, blood glucose and HbA_{1c} in control and diabetic mice

Strain	Group	Duration (m)	n	Weight (g)	Glucose (mg/ml)	HbA_{1c} (%)	HbA_{1c} (mmol/mol)
B6	Control	2	32	34 + 2.1	146.2 + 25.4	3.3 + 0.1	13
	Diabetic	2	31	29 + 1.4	527 + 30.5	7.9 + 0.3	63
$CD40^{-/-}$	Control	2	26	34.5 + 1.8	172.3 + 8.7	3.6 + 0.1	16
	Diabetic	2	30	26.4 + 0.9	556.3 + 17.2	8.9 + 0.5	74
Trg-Ctr	Control	2	38	32.8 + 1.2	143.4 + 7.5	3.3 + 0.1	13
	Diabetic	2	38	25 + 0.7	537.2 + 11.5	8 + 0.3	64
Trg-CD40	Control	2	40	33.3 + 1.2	186.6 + 17.1	3.4 + 0.2	14
	Diabetic	2	69	26.6 + 0.9	533.4 + 21.7	8.4 + 0.2	68
$P2X7^{-/-}$	Control	2	9	35 + 2	140.3 + 8.7	3 + 0.1	9
	Diabetic	2	11	24 + 1	500 + 15.3	7 + 0.2	53
B6	Control	8	7	43 + 2.0	154.5 + 9.2	3.5 + 0.1	15
	Diabetic	8	8	30.4 + 1.2	452 + 21.6	10.3 + 0.8	89
Trg-Ctr	Control	8	10	44.7 + 2.0	150.3 + 5.2	3.5 + 0.1	15
	Diabetic	8	6	29 + 0.5	479.3 + 25.3	10.8 + 0.3	95
Trg-CD40	Control	8	6	46.5 + 1.3	168 + 10.5	3.5 + 0.1	15
	Diabetic	8	6	27.5 + 0.5	477.8 + 33.9	11.5 + 0.2	102