Supporting information

Table S1. Antibodies used for western blot analysis.

Target protein	Company	Catalog number	Concentration
Pfkfb3	Abcam	ab181861	1:1000
Hif1a	R&D Systems	AF1935-SP	1:1000
Hif2a	Novus	NB100-122	1:500
P-p65	Cell Signaling Technology	3033S	1:1000
P65	Cell Signaling Technology	8242S	1:1000
Il1b	R&D Systems	AF-401-NA	1:1000
β-actin	Santa Cruz Biotechnology	sc-47778	1:1000
Anti-mouse IgG, HRP-linked Antibody	Cell Signaling Technology	7076S	1:2000
Anti-rabbit IgG, HRP-linked antibody	Cell Signaling Technology	7074S	1:2000
mouse anti-goat IgG-HRP	Santa Cruz Biotechnology	sc-2354	1:2000

Table S2. Antibodies used for immunostaining analysis.

Target protein	Company	Catalog number	Concentration
isolectin-B4	Invitrogen	I21411	1:100
F4/80	Abcam	ab6640	1:100
Pfkfb3	Abcam	ab181861	1:100
Arg1	Sigma	AV45673	1:100
Il1b	Abcam	ab9722	1:100
Hif1a	R&D Systems	AF1935-SP	1:100
Hif2a	Novus	NB100-122	1:100
HK1	Proteintech	19662-1-AP	1:100
Glut1	Abcam	ab115730	1:100
Pkm2	Cell Signaling Technology	4053	1:100
P-p65	Cell Signaling Technology	3033S	1:100
Alexa Fluor 488-conjugated goat anti-rat secondary antibody	Invitrogen	A11006	1:200
Alexa Fluor 594-conjugated goat anti-rabbit secondary antibody	Invitrogen	A11012	1:200
Alexa Fluor 594-conjugated goat anti-rat secondary antibody	Invitrogen	A11007	1:200
Alexa Fluor 647-conjugated goat anti-rabbit secondary antibody	Invitrogen	A21244	1:200
Alexa Fluor 594-conjugated donkey anti-goat secondary antibody	Invitrogen	A11058	1:200
Alexa Fluor 647-conjugated donkey anti-rat secondary antibody	Invitrogen	A78947	1:200

Table S3. Primer sets used for qRT-PCR (F: forward primer; R: reverse primer)

Target gene	Sequence
mouse*18S ribosomal RNA*	F: 5'- CTTAGAGGGACAAGTGGCG -3' R: 5'- ACGCTGAGCCAGTCAGTGTA -3'
mouse *Rpl13a*	F:5'- GAGGTCGGGTGGAAGTACCA -3' R:5'- TGCATCTTGGCCTTTTCCTT -3'
mouse *Slc2a1*	F: 5'- GCAGTTCGGCTATAACACTGG -3' R: 5'- GCGGTGGTTCCATGTTTGATTG -3'
mouse *Hk1*	F: 5'- AACGGCCTCCGTCAAGATG -3' R: 5'- GCCGAGATCCAGTGCAATG -3'
mouse *Hk2*	F: 5'- ATGATCGCCTGCTTATTCACG -3' R: 5'- CGCCTAGAAATCTCCAGAAGGG -3'
mouse *Gpi*	F: 5'- CTCAAGCTGCGCGAACTTTTT -3' R: 5'- GGTTCTTGGAGTAGTCCACCAG -3'
mouse *Pfkfb3*	F: 5'- GATCTGGGTGCCCGTCGATCACCG -3' R: 5'- CAGTTGAGGTAGCGAGTCAGCTTC -3'
mouse *Aldoa*	F:5'- AGTCCACCGGAAGCATTGC -3' R:5'- CAGCCCCTGGGTAGTTGTC -3'
mouse *Pgk1*	F:5'- ATGTCGCTTTCCAACAAGCTG -3' R:5'- GCTCCATTGTCCAAGCAGAAT -3'
mouse *Eno1*	F:5'- TGCGTCCACTGGCATCTAC -3' R:5'- CAGAGCAGGCGCAATAGTTTTA -3'
mouse *Pdk1*	F:5'- GGACTTCGGGTCAGTGAATGC -3' R:5'- TCCTGAGAAGATTGTCGGGGA -3'
mouse *Ldha*	F:5'- ACATTGTCAAGTACAGTCCACAC -3' R:5'- TTCCAATTACTCGGTTTTTGGGA -3'
mouse *Ldhb*	F:5'- TGCGTCCGTTGCAGATGAT-3' R:5'- TTTCGGAGTCTGGAGGAACAA-3'
mouse *Pkm2*	F:5'- AGGATGCCGTGCTGAATG -3' R:5'- TAGAAGAGGGGCTCCAGAGG -3'
mouse *Cd163*	F:5'- GGTGGACACAGAATGGTTCTTC -3' R:5'- CCAGGAGCGTTAGTGACAGC -3'
mouse *Cd206*	F:5'- TCTTTGCCTTTCCCAGTCTCC -3' R:5'- TGACACCCAGCGGAATTTC -3'
mouse *Arg1*	F:5'- CAGAAGAATGGAAGAGTCAG -3' R:5'- CAGATATGCAGGGAGTCACC -3'
mouse *Retnla*	F:5'- CCCTGCTGGGATGACTGCTA -3' R:5'- TCCACTCTGGATCTCCCAAGA -3'
mouse *Mgl1*	F:5'- CAGAATCGCTTAGCCAATGTGG -3' R:5'- TCCCAGTCCGTGTCCGAAC -3'
mouse *Mgl2*	F:5'- TTCAAGAATTGGAGGCCACT -3' R:5'- CAGACATCGTCATTCCAACG -3'
mouse *Irf4*	F:5'- TCCGACAGTGGTTGATCGAC -3' R:5'- CCTCACGATTGTAGTCCTGCTT -3'
mouse *Chi3l3*	F:5'- GGAGTAGAGACCATGGCACTGAAC -3' R:5'- GACTTGCGTGACTATGAAGCATTG -3'
mouse *Il10*	F:5'- GCTATGCTGCCTGCTCTTACT -3' R:5'- CCTGCTGATCCTCATGCCA -3'
mouse *Cd11c*	F:5'- TCGTTGGCCTCTAACGAGCTTTCT -3' R:5'- AGGATAACATGGAAGCACGGACCA -3'
mouse *Cd80*	F:5'- ACCCCCAACATAACTGAGTCT -3' R:5'- TTCCAACCAAGAGAAGCGAGG -3'

mouse *Il6*	F:5'- GTTCTCTGGGAAATCGTGGA -3' R:5'- TGTACTCCAGGTAGCTATGG -3'
mouse *Il1b*	F:5'- TGTCTTGGCCGAGGACTAAGG -3' R:5'- TGGGCTGGACTGTTTCTAATGC -3'
mouse *Tnfa*	F:5'- ACGGCATGGATCTCAAAGAC -3' R:5'- AGATAGCAAATCGGCTGACG -3'
mouse *Nos2*	F:5'- CAGCTGGGCTGTACAAACCTT -3' R:5'- CATTGGAAGTGAAGCGTTTCG -3'
mouse *Cxcl10*	F:5'- GAGCCTATCCTGCCCACG -3' R:5'- GGAGCCCTTTTAGACCTT -3'
mouse *Angptl4*	F:5'- CATCCTGGGACGAGATGAACT -3' R:5'- TGACAAGCGTTACCACAGGC -3'
mouse *Tgfb1*	F:5'- ATGGTGGACCGCAACAAC -3' R:5'- CCAAGGTAACGCCAGGAA -3'
mouse *Vegf*	F:5'- TCACCAAAGCCAGCACATAGGAGA -3' R:5'- TTTCTCCGCTCTGAACAAGGCTCA -3'
mouse *Fgf2*	F:5'- GCGACCCACACGTCAAACTA -3' R:5'- TCCCTTGATAGACACAACTCCTC -3'
mouse *Hbegf*	F:5'- CGGGGAGTGCAGATACCTG -3' R:5'- TTCTCCACTGGTAGAGTCAGC -3'
mouse *Hgf*	F:5'- ATGTGGGGGACCAAACTTCTG -3' R:5'- GGATGGCGACATGAAGCAG -3'
mouse *Pgf*	F:5'- GTCTGCTGGGAACAACTCAACA -3' R:5'- CACCTCATCAGGGTATTCATCCA -3'
mouse *Mmp9*	F:5'- TTGAAGTCTCAGAAGGTGGAT -3' R:5'- GCAGGAGGTCGTAGGTCAC -3'
mouse *Mmp12*	F:5'- AAAGTGGGTTGTAGCATTGC -3' R:5'- AGAAGGCAGACCAGGACAC -3'
mouse *Mmp2*	F:5'- CCTGGACCCTGAAACCGTG -3' R:5'- TCCCCATCATGGATTCGAGAA -3'
mouse *Vegf*	F:5'- TCACCAAAGCCAGCACATAGGAGA -3' R:5'- TTTCTCCGCTCTGAACAAGGCTCA -3'

Table S4. Antibodies used for FACS analysis.

Target protein	Company	Catalog number	Concentration
PerCP-Cy5.5 anti-mouse CD11b	BD Biosciences	561114	2 μg/ml
PE-anti-mouse F4/80	BD Biosciences	565410	3 μg/ml
FITC anti-mouse CD206	Biolegend	141703	6 μg/ml
BV510 anti-mouse CD11c	BD Biosciences	562949	3 μg/ml

Table S5. Demographic Information of Control Patients.

Identifier	Age	Sex	Race	Eye	Sample	Setting of Sample Collection	Prior Anti VEGF treatment	Time since last anti-VEGF
Patient 1	45	F	White	OS	Aqueous	Cataract Surgery	N/A	N/A
Patient 2	71	M	African American	OD	Aqueous	Cataract Surgery	N/A	N/A
Patient 3	47	F	White	OD	Aqueous	Cataract Surgery	N/A	N/A
Patient 4	67	F	White	OS	Aqueous	Cataract Surgery	N/A	N/A
Patient 5	52	M	White	OD	Aqueous	Cataract Surgery	N/A	N/A
Patient 6	80	M	White	OD	Aqueous	Cataract Surgery	N/A	N/A
Patient 7	86	M	Hispanic	OS	Aqueous	Cataract Surgery	N/A	N/A
Patient 8	68	M	White	OS	Aqueous	Cataract Surgery	N/A	N/A
Patient 9	82	M	White, Other	OS	Aqueous	Cataract Surgery	N/A	N/A
Patient 10	76	F	White	OS	Aqueous	Cataract Surgery	N/A	N/A
Patient 11	79	F	White	OD	Aqueous	Cataract Surgery	N/A	N/A
Patient 12	80	F	White	OD	Aqueous	Cataract Surgery	N/A	N/A
Patient 13	70	F	African American	OD	Aqueous	Cataract Surgery	N/A	N/A
Patient 14	64	F	White	OD	Aqueous	Cataract Surgery	N/A	N/A
Patient 15	79	M	White	OD	Aqueous	Cataract Surgery	N/A	N/A

Table S6. Demographic Information of Patients with new Choroidal Neovascularization (no prior activity or treatment for at least 6 months).

Identifier	Age	Sex	Race	Eye	Sample	Setting of Sample Collection	Prior Anti VEGF Treatment	Injection History
Patient 1	81	M	White	OS	Aqueous	Intravitreal Injection	No	N/A
Patient 2	67	F	White	OS	Aqueous	Intravitreal Injection	No	N/A
Patient 3	74	F	White	OS	Aqueous	Intravitreal Injection	No	N/A
Patient 4	74	F	White	OD	Aqueous	Intravitreal Injection	No	N/A
Patient 5	71	M	White	OD	Aqueous	Intravitreal Injection	Yes	Last injection 3 years prior; quiescent until resumed treatment
Patient 6	79	M	White	OS	Aqueous	Intravitreal Injection	No	N/A
Patient 7	83	F	White	OD	Aqueous	Intravitreal Injection	No	N/A
Patient 8	77	F	White	OD	Aqueous	Intravitreal Injection	No	N/A
Patient 9	77	F	White	OD	Aqueous	Intravitreal Injection	Yes	Last injection 8 months prior; quiescent until resumed treatment
Patient 10	89	F	White	OS	Aqueous	Intravitreal Injection	No	N/A
Patient 11	56	F	White	OS	Aqueous	Intravitreal Injection	No	N/A
Patient 12	91	F	White	OS	Aqueous	Intravitreal Injection	No	N/A

Table S7. Demographic Information of Patients with Choroidal Neovascularization undergoing ongoing treatment (within 3 months).

Identifier	Age	Sex	Race	Eye	Sample	Setting of Sample Collection	Prior Anti VEGF treatment	Injection History
Patient 13	89	F	White	OD	Aqueous	Intravitreal Injection	Yes	4th injection; last injection 2 months prior
Patient 14	78	F	White	OD	Aqueous	Intravitreal Injection	Yes	3rd injection; last injection 2 months prior
Patient 15	87	F	White	OD	Aqueous	Intravitreal Injection	Yes	2nd injection; last injection 2 months prior
Patient 16	69	F	White	OS	Aqueous	Intravitreal Injection	Yes	2nd injection; last injection 1 month prior
Patient 17	90	F	White	OS	Aqueous	Intravitreal Injection	Yes	2nd injection; last injection 2 months prior

Supplementary Figures and Figure Legends

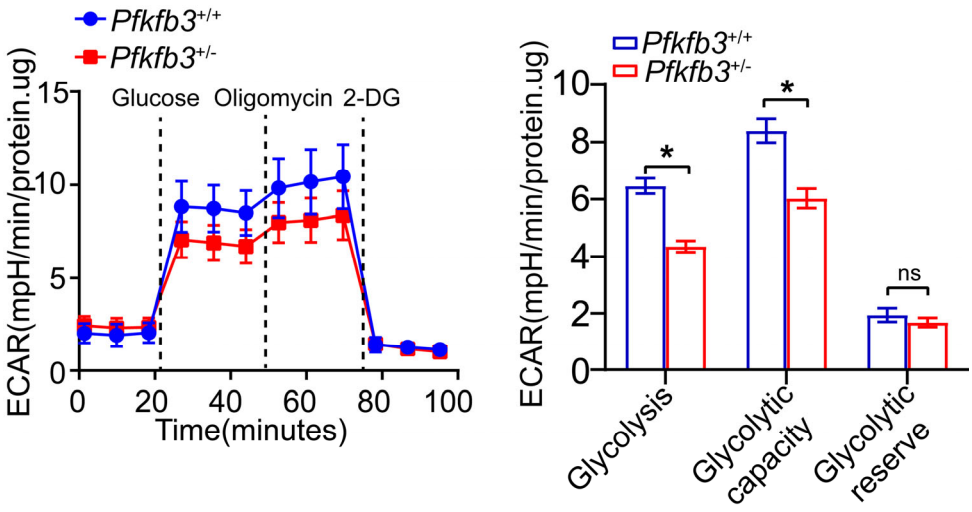

Fig. S1. Heterozygous *Pfkfb3* deficiency in mice decreased the glucose-induced glycolysis in BMDMs. ECAR analysis shows glycolytic function in *Pfkfb3*$^{+/+}$ and *Pfkfb3*$^{+/-}$ BMDMs (n=8-9). Data were presented as means ± SEM. Statistical significance was conducted by unpaired two-tailed Student's *t* test or unpaired two-tailed Student's *t* test with Welch's correction. *$p < 0.05$ was considered significant.

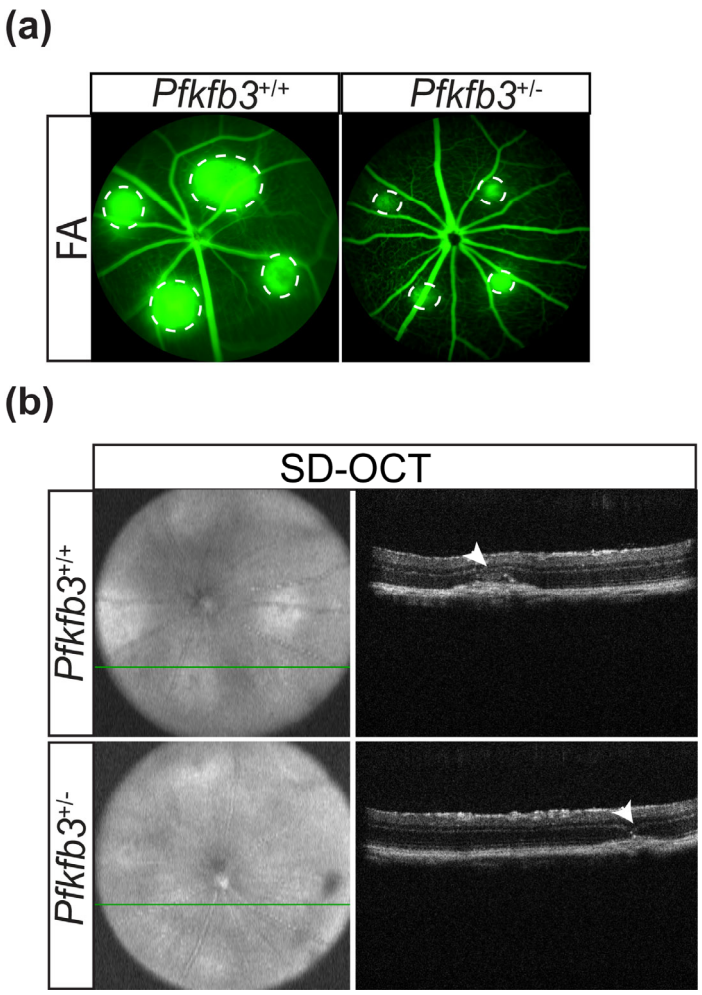

Fig. S2. Heterozygous *Pfkfb3* deficiency in mice decreased the severity of vascular leakage and CNV thickness from CNV lesions. (a) Representative images of fluorescein leakage from CNV lesions in *Pfkfb3*$^{+/+}$ and *Pfkfb3*$^{+/-}$ mice on day 7 after CNV induction. **(b)** Representative cross-sectional images of CNV obtained through SD-OCT in *Pfkfb3*$^{+/+}$ and *Pfkfb3*$^{+/-}$ mice on day 7 after CNV induction.

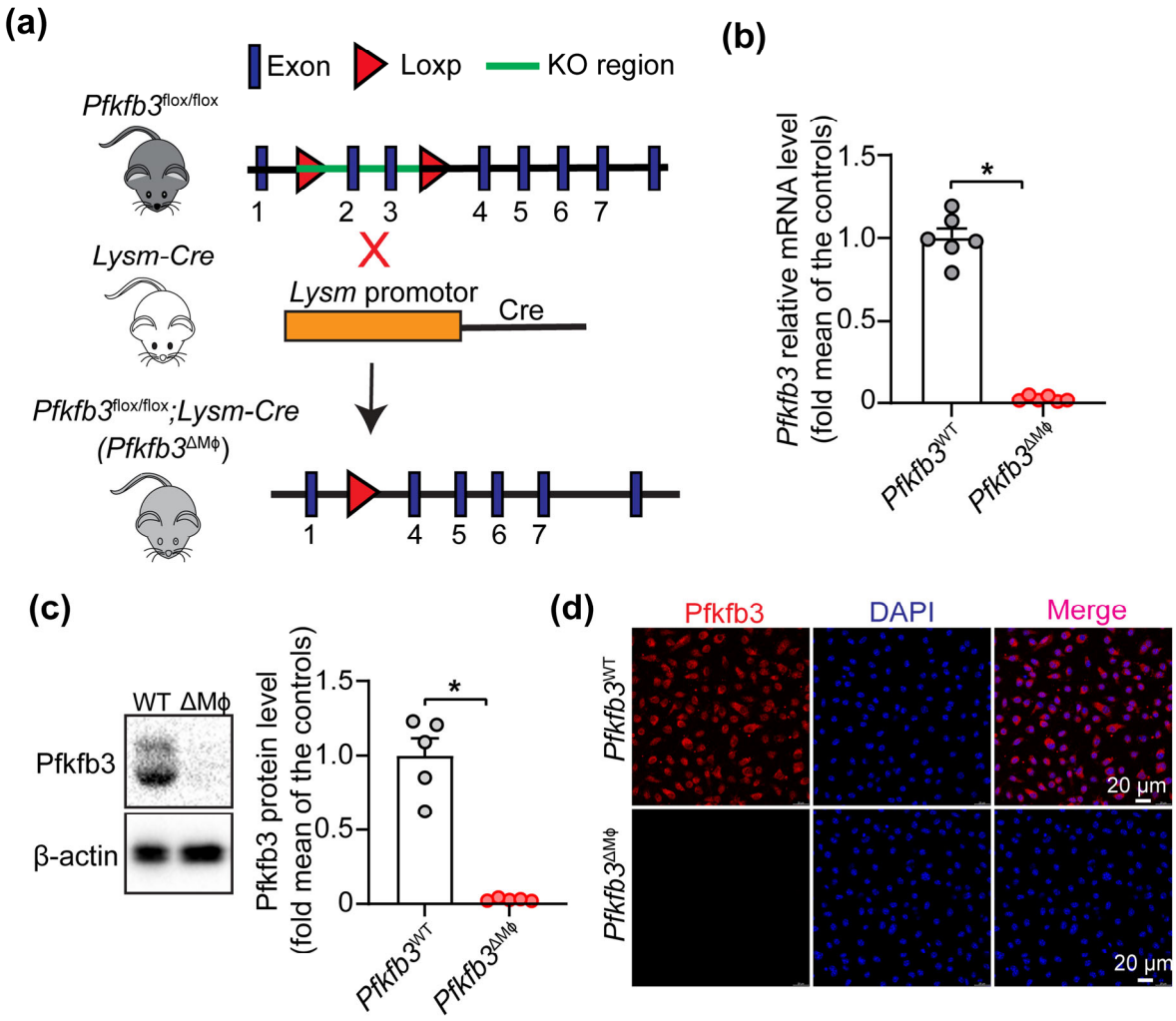

Figure S3. Generation of myeloid-specific *Pfkfb3* knock out mice. (a) Schematic diagram of strategy to generate myeloid-specific *Pfkfb3* KO mice. **(b)** qRT-PCR analysis of mRNA level of *Pfkfb3* in BMDMs from *Pfkfb3*^{WT} and *Pfkfb3*$^{\Delta M\varphi}$ mice (n=6 mice per group). **(c)** Western blot analysis and densitometric quantification of *Pfkfb3* in BMDMs from *Pfkfb3*^{WT} and *Pfkfb3*$^{\Delta M\varphi}$ mice (n=5 mice per group). **(d)** Representative Pfkfb3 immunostaining images in BMDMs from *Pfkfb3*^{WT} and *Pfkfb3*$^{\Delta M\varphi}$ mice (Scale bar =20 μm). Data were presented as means ± SEM. Statistical analysis was conducted by unpaired two-tailed Student's *t* test with Welch's correction. $P < 0.05$ was considered significant.

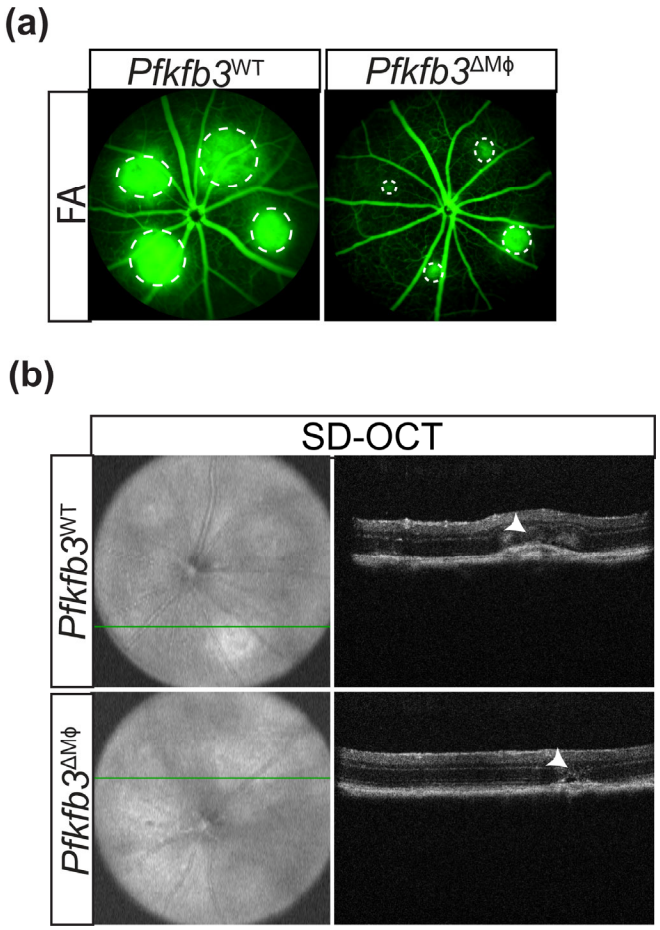

Fig. S4. Myeloid-specific *Pfkfb3* deficiency in mice decreased the severity of vascular leakage and CNV thickness from CNV lesions. (a) Representative images of fluorescein leakage from CNV lesions in *Pfkfb3*^{WT} and *Pfkfb3*$^{\Delta M\phi}$ mice on day 7 after CNV induction. **(b)** Representative cross-sectional images of CNV obtained through SD-OCT in *Pfkfb3*^{WT} and *Pfkfb3*$^{\Delta M\phi}$ mice on day 7 after CNV induction.

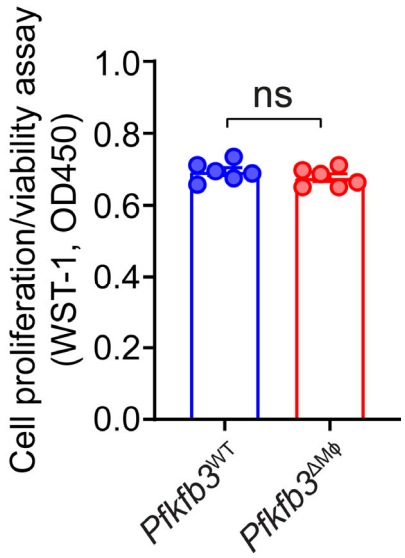

Fig. S5. *Pfkfb3* loss had no effect on the cell viability of MΦ. Cell proliferation/viability were measured in *Pfkfb3*^{WT} and *Pfkfb3*$^{\Delta M\phi}$ BMDMs by WST-1 cell proliferation/viability assay. n = 6. Data were presented as means ± SEM. Statistical significance was conducted by unpaired two-tailed Student's *t* test. * $p < 0.05$ was considered significant, ns, no significance.

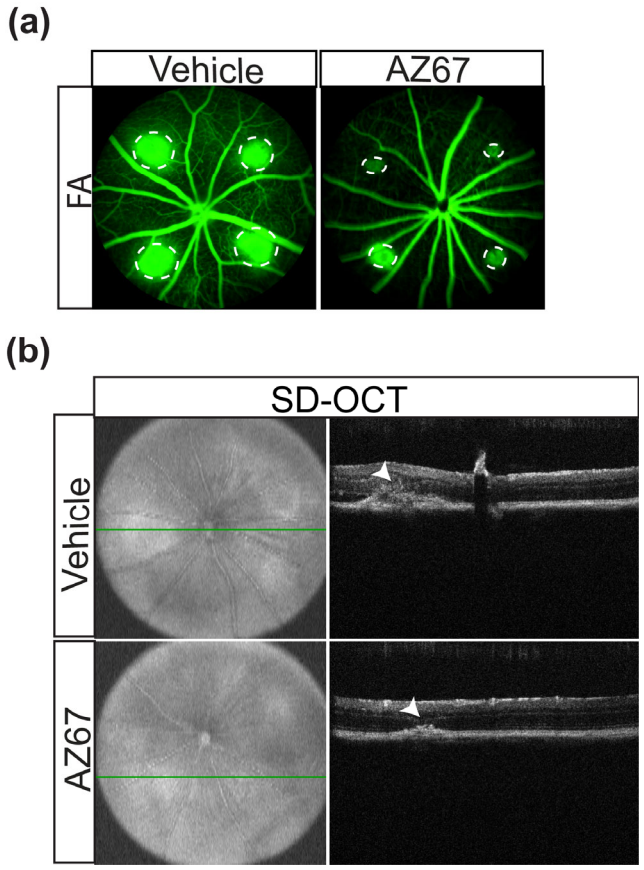

Fig. S6. AZ67 administration decreased the severity of vascular leakage and CNV thickness from CNV lesions. (a) Representative images of fluorescein leakage from CNV lesions vehicle- or AZ67-treated WT mice on day 7 after CNV induction. **(b)** Representative cross-sectional images of CNV obtained through SD-OCT in vehicle- or AZ67-treated WT mice on day 7 after CNV induction.