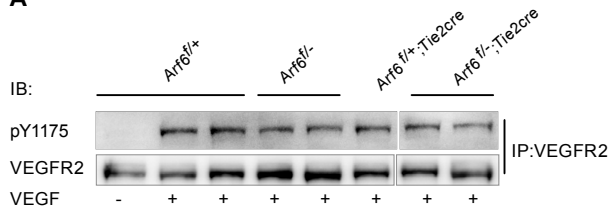
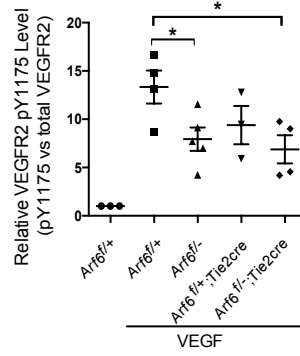
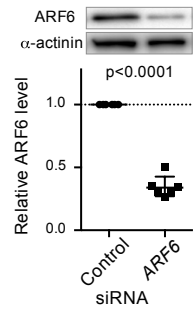
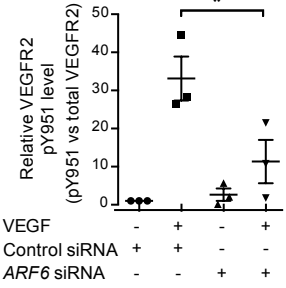
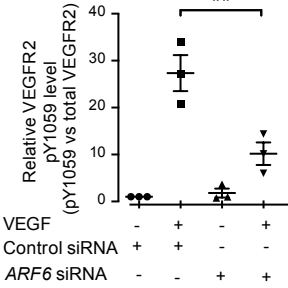
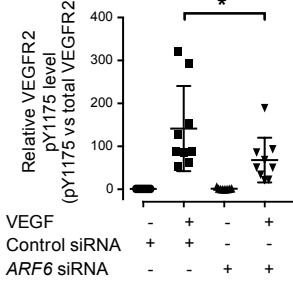
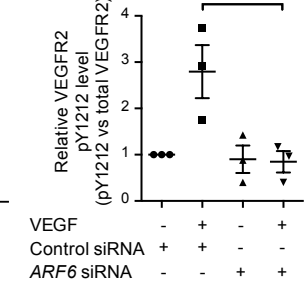
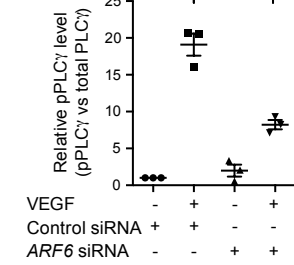
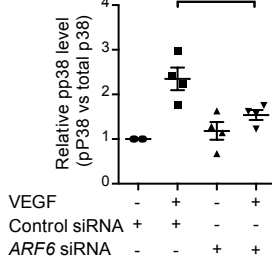
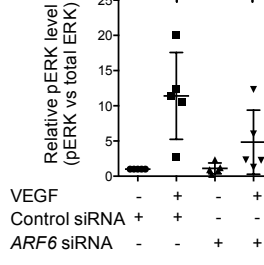
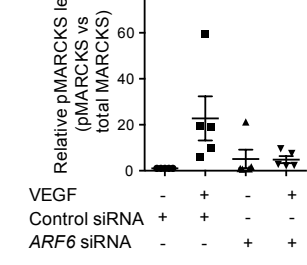
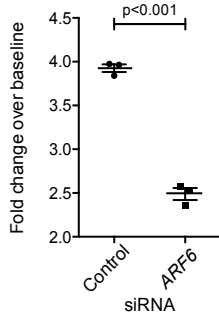
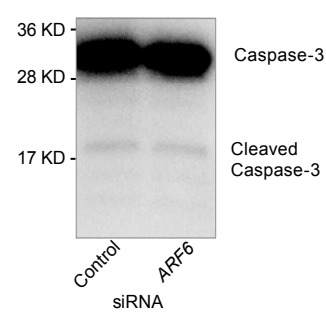
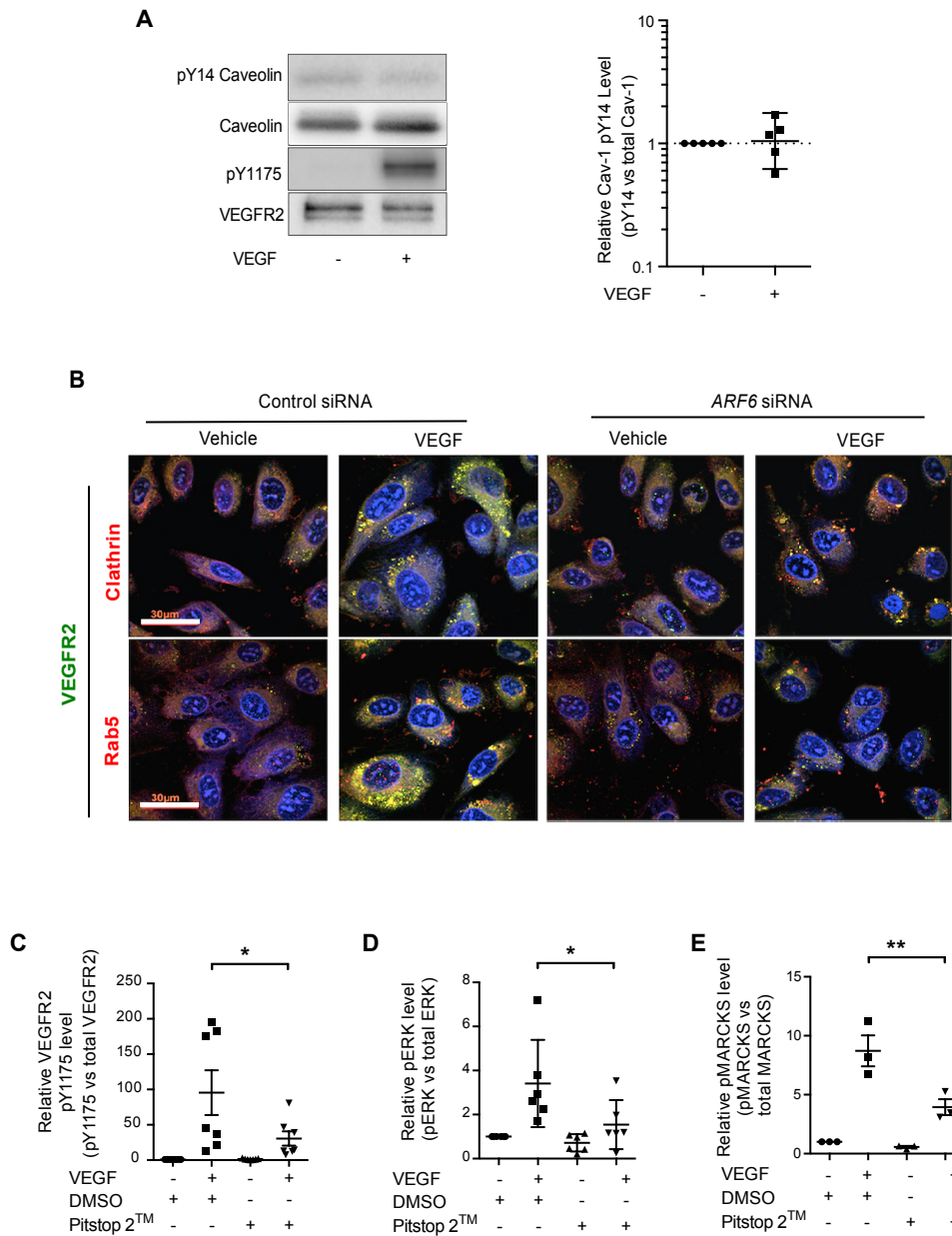


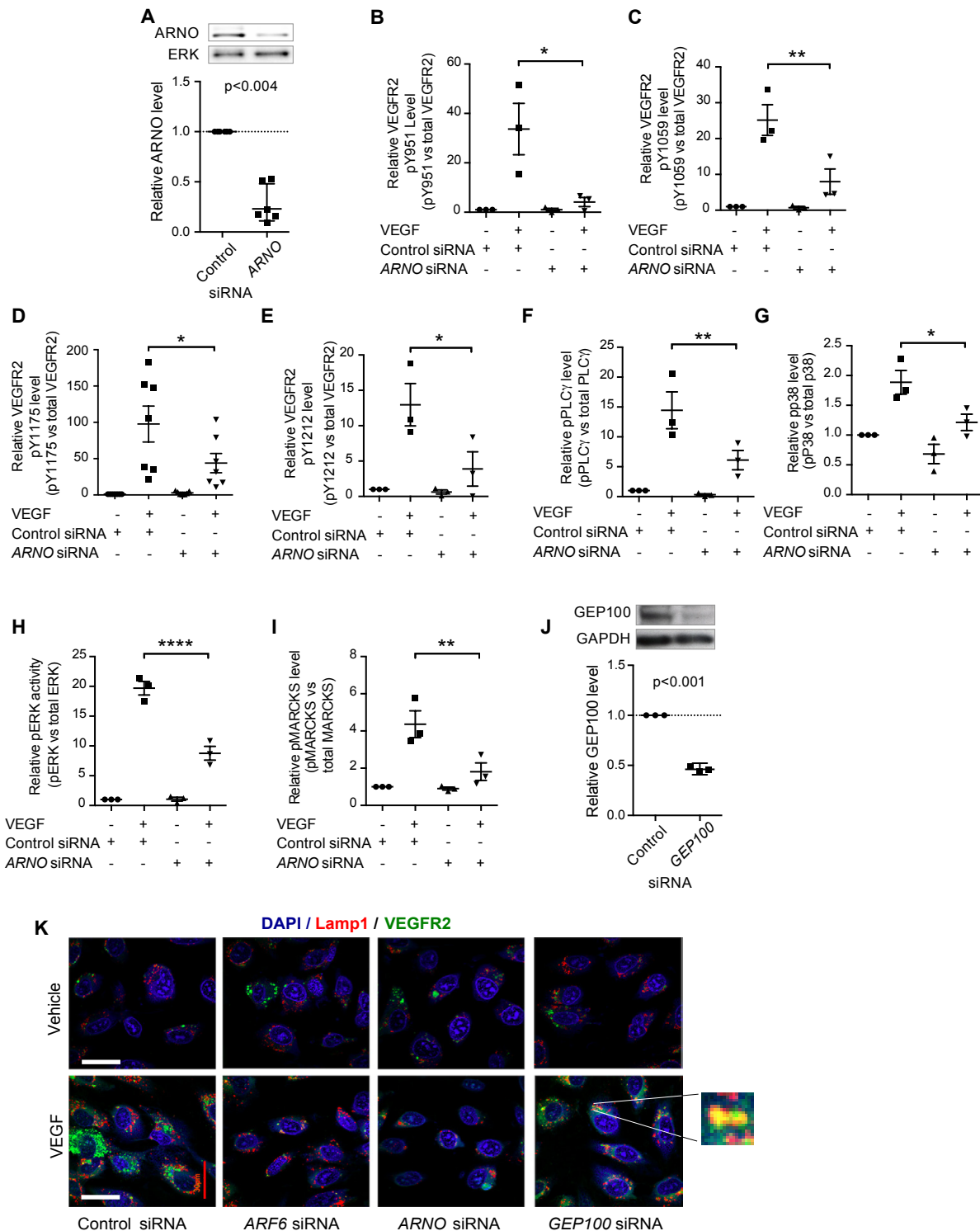
**Supplemental Figure 1. Generation and validation of *Arf6* endothelial knockout mice.** (A) Eye lysates from diabetic human patients immunoblotted for ARF6. The average age of diabetic group is  $74.60 \pm 9.099$  and control group is  $68.00 \pm 17.79$  (there is no significant difference in age between the two groups). (B) Evans blue permeability in mice treated with saline or STZ. (C) Schematic of modified *Arf6* alleles. (D) Validation of *Arf6* knockout from lysates of aortic endothelial cells. (E) Retinal vessels of *Arf6* knockout mice and other genotypes as well (8 weeks). Scale bar = 500  $\mu$ m. (G) Blood glucose levels of *Arf6<sup>f/-</sup>; Tie2cre* mice and their siblings. In panel A (n=5), an unpaired Welch's t-test (two-tailed) was used to assess statistical significance. In panel B (n=2 or 4), a Student's t-test (two-tailed) was used to assess statistical significance; \*p<0.05. Error bars represent SEM.

**A****B****C****D****E****F****G****H****I****J****K****L****M**

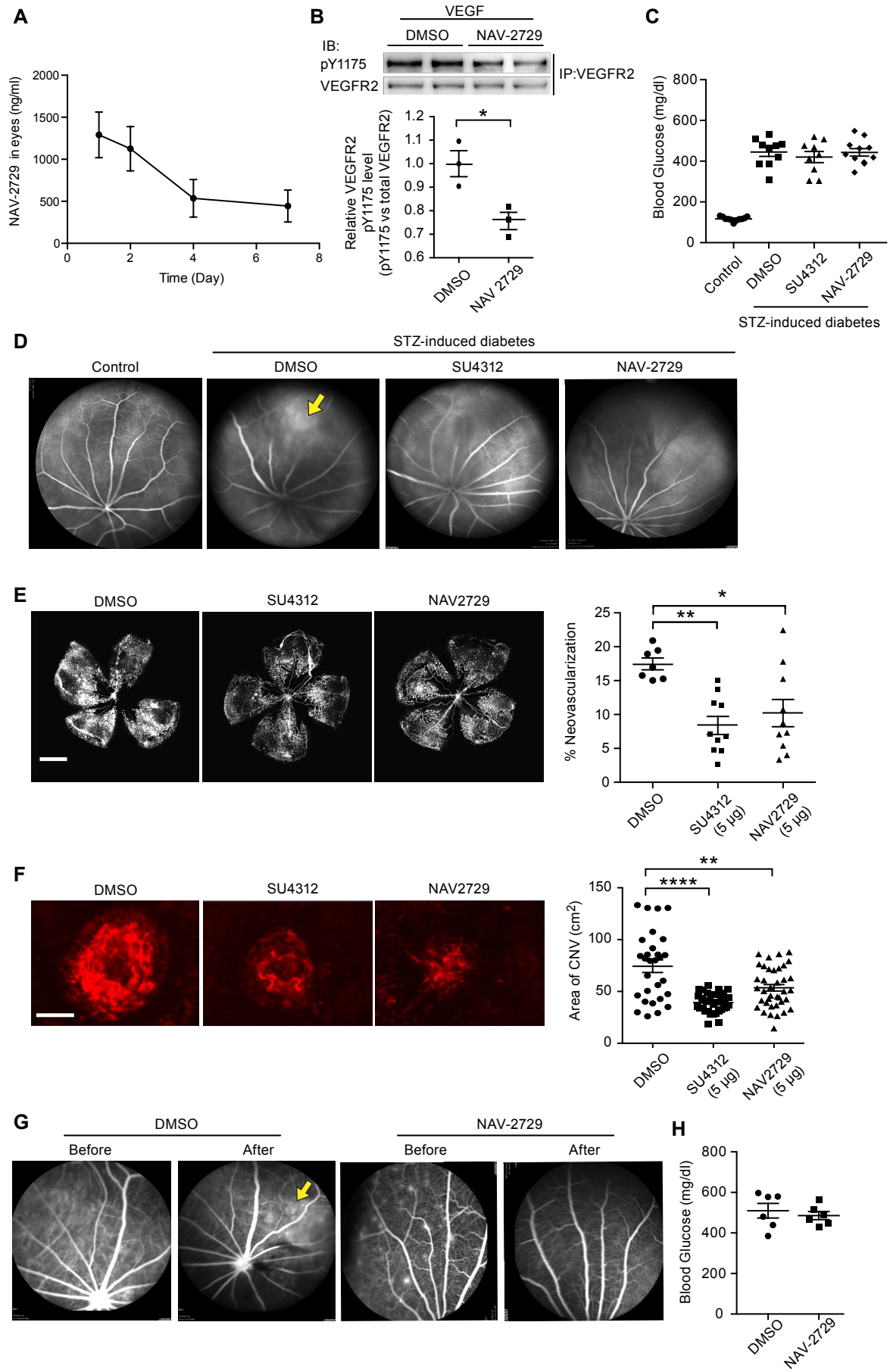
**Supplemental Figure 2. Supporting data and quantification of immunoblots in Figure 2.** (A,B) *Arf6*<sup>-/-</sup>; *Tie2cre* mice and their siblings were give VEGF through the tail vein for 1 min and two eyes from each mouse were harvested and pooled. VEGFR2 was immunoprecipitated, and the precipitate was immunoblotted using antibodies for pVEGFR2 (Y1175). In panel A, groupings of images are different parts of the same gel. (C) Cell lysates from HRECs transfected with control siRNA or *ARF6* siRNA immunoblotted with anti-ARF6 antibody to confirm knockdown. (D-G) Quantification of immunoblots represented in Figure 2A. (H-K) Quantification of immunoblots represented in Figure 2B. (L) HRECs transfected with control siRNA or *ARF6* siRNA were assayed for proliferation. (M) Cell lysates from HRECs transfected with control siRNA or *ARF6* siRNA immunoblotted with anti-Caspase-3 antibody to detect cell apoptosis. In panels B ( $n \geq 3$ ) and D-K ( $n=3$  to  $n=9$ ), one-way ANOVA with Dunnett's multiple comparisons tests was used to assess statistical significance; error bars represent SEM; \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\*\* $p < 0.0001$ . In panel C ( $n=6$ ), geometric means and 95% confidence intervals (error bars) of the ratios were plotted on a logarithmic scale. Statistical significance was assessed using the ratio paired t test (two-tailed) on non-normalized data, and the p values are shown in the graph. In panel L ( $n=3$ ), a Student's t-test (two-tailed) was used to assess statistical significance; error bars represent SEM.



**Supplemental Figure 3. Supporting data and quantification of immunoblots in Figure 3.** (A) Cell lysates from HRECs treated with VEGF and immunoblotted for pY14 Caveolin or Caveolin. Phosphorylation of VEGFR2 at Y1175 was used as a control to confirm VEGF is functioning in the assay. (B) ARF6 siRNA- or Control siRNA-treated HRECs were stimulated with VEGF for 5 min and assayed for co-localization of internalized VEGFR2 with Clathrin and Rab5 (scar bar: 30  $\mu$ m; Original magnification,  $\times$ 1,200). The bottom row of panel B shows the Z-stacked images for Figure 3C. (C-E) Quantification of immunoblots represented in Figure 3D. In panel A (n=5), geometric means and 95% confidence intervals (error bars) of the ratios were plotted on a logarithmic scale. The differences were not significant using a ratio paired t test (two-tailed) on non-normalized data. In panel C-E (n=3 to n=7), one-way ANOVA with Dunnett's multiple comparisons test was used to assess statistical significance; error bars represent SEM; \*p<0.05, \*\*p<0.01



**Supplemental Figure 4. Supporting data and quantification of immunoblots in Figure 4 and 5.** (A) Cell lysates from HRECs transfected with control siRNA or ARNO siRNA immunoblotted with anti-ARNO antibody to confirm knockdown. (B-E) Quantification of immunoblots represented in Fig. 4C. (F-I) Quantification of immunoblots represented in Fig. 4D. (J) Cell lysates from HRECs transfected with control siRNA or GEP100 siRNA immunoblotted with anti-GEP100 antibody to confirm knockdown. (K) The Z-stacked image for Figure 5F (scar bar: 30  $\mu$ m; Original magnification,  $\times 1,200$  and additional  $\times 7$  (inset). In panels A (n=6) and J (n=3), geometric means and 95% confidence intervals (error bars) of the ratios were plotted. Statistical significance was assessed using the ratio paired t test (two-tailed) on non-normalized data, and the p value is shown in the graph. In panel B-I (n=3 or 7), one-way ANOVA with Dunnett's multiple comparisons test was used to assess statistical significance; error bars represent SEM; \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\*\* $p < 0.0001$ .



**Supplemental Figure 5. Supporting data for Figure 7.** (A) 50 ng of NAV-2729 was injected into each eye of a mouse and both eyes were harvested and pooled for pharmacokinetic analysis. (B) NAV-2729 was injected into each mouse eye 30 min before injecting the tail vein with VEGF. Eyes from each mouse were harvested 1 min after VEGF injection, and pooled. VEGFR2 was immunoprecipitated, and the precipitate was immunoblotted using antibodies for pVEGFR2 (Y1175). (C) Blood glucose levels of mice used for Figure 7D. (D) Fluorescein angiography was performed in SU4312- or NAV-2729-treated and untreated diabetic mice. (E) Retinal flat mounts were prepared from neonatal mice subjected to OIR, stained with fluorescent isolectin and analyzed by fluorescence microscopy. Scale bar: 1000  $\mu\text{m}$ . (F) Mice were subjected to laser-induced choroidal neovascularization. Choroidal flat mounts were prepared, stained with fluorescent isolectin and analyzed by confocal microscopy. Scale bar: 100  $\mu\text{m}$ . (G) Fluorescein angiography was performed in NAV-2729-treated and untreated diabetic rats. Eyes from same animal before and after different treatment with DMSO or NAV-2729 were represented. (H) Blood glucose levels of rats used for Figure 7E-7G. In panel B (n=3), a Student's t-test (two-tailed) was used to assess statistical significance; \*p<0.05. In Panel E (n>7) and F (n>29) one-way ANOVA with Dunnett's multiple comparisons tests was used to assess statistical significance; \*p<0.05, \*\*p<0.01, \*\*\*\*p<0.0001. Error bars represent SEM.

**Supplemental Table 1. Patient Information**

Patient	Number of eyes used in study	Age	Sex	PMH	Ocular Hx	Diabetes Dx	Cause of Death
14-0620-101, 201	2	60	Female	Parkinson's disease, Alzheimer's disease, rapid progressing with dementia with Lewy bodies, depression, anxiety, osteoarthritis, hypothyroidism, hysterectomy.	None	No	Aspiration
14-0580-101	1	47	Female	Chronic back pain, metastatic breast cancer, tobacco use.	None	No	Metastatic breast cancer
14-0213-101	1	84	Female	a-fib, HTN, peripheral neuropathy, chronic back problems, cholecystectomy, hysterectomy, spinal fusion, R total hip replacement, rheumatic fever	None	No	Respiratory NOS
13-1193-101	1	89	Female	Dementia as well as poor hearing, coronary artery disease, hypothyroidism, HTN, and hyperlipidemia.	Bilateral cataract surgery	No	Myocardial Infarction
14-0610-101,201	2	72	Male	Multiple pulmonary emboli, diabetes, cerebral palsy with left side weakness, asthma, hypertension, cerebral vascular accident, osteoarthritis, obesity, colonic polyp, degenerative arthritis, type IV hyperlipoproteinemia, dementia, asthma, tonsillectomy, appendectomy.	Diabetic retinopathy, cataracts	Yes	Pulmonary Embolism
14-0582-101	1	62	Female	HTN, depression, knee surgery, hernia surgery, COPD, DM, CHF- d/t Fen- Phen, bladder surgery, appendix, smoker.	Lasik surgery	Yes	Pneumonia
14-0039-101	1	83	Male	A-fib, coronary artery disease, congestive heart failure, hyperlipidemia, bladder cancer, transitional cell carcinoma, muscle invasive cancer, herniated disk, degenerative joint disease, type 2 diabetes, kidney infection, pilonidal cyst removed from tailbone, stoma with subsequent surgeries.	Bilateral cataract surgery	Yes	Sudden cardiac event
14-0237-101	1	84	Female	DM, reactive airway disease, Rheumatoid arthritis, arterial HTN, dyslipidemia, GERD, CAD, CHF, paroxysmal atrial fibrillation, RLS, total right knee arthroplasty, pacemaker placement, peripheral arterial disease, atherosclerotic cardiovascular disease, superior rectus muscle resection both sides, hysterectomy, cholecystectomy, appendectomy, carotid artery stents, osteoarthritis, cervicalgia, RLS.	Intraocular lens implant, bilateral	Yes	Sepsis

**Supplemental Table 2. Urine test by Urine Reagent Panel Strips (IVD grade)**

	<i>Arf6<sup>f/+</sup></i>	<i>Arf6<sup>f/+</sup>;Tie2cre</i>	<i>Arf6<sup>f/-</sup>;Tie2cre</i>	<i>Normal range in mice</i>
<b>Leukocyte</b>	(-)	(-)	(-)	(-)
<b>Nitrite</b>	(-)	(-)	(-)	(-)
<b>Urobilinogen (mg/dl)</b>	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ~ 1.0
<b>pH</b>	5.7 ± 0.6	6.0 ± 0.0	5.3 ± 0.6	5 ~ 6
<b>Blood</b>	(-)	(-)	(-)	(-)
<b>Specific gravity</b>	1.028 ± 0.003	1.030 ± 0.000	1.030 ± 0.000	N.A.
<b>Ketone (mm)</b>	5 ± 0	2 ± 2	5 ± 0	2.5 ~ 10
<b>Bilirubin</b>	(-)	(-)	(-)	(-)
<b>Glucose</b>	(-)	(-)	(-)	(-)

**Supplemental Table 3. Complete Blood Count by Automatic Hemacytometer (Veterinarian grade)**

	<i>Arf6<sup>f/+</sup></i>	<i>Arf6<sup>f/+</sup>;Tie2cre</i>	<i>Arf6<sup>f/-</sup>;Tie2cre</i>	<b>Normal range in mice</b>
<b>White blood cell (10<sup>3</sup>/μL)</b>	4.5 ± 1.4	5.6 ± 3.6	4.5 ± 2.5	2.6 ~ 10.1
<b>Granulocyte (10<sup>3</sup>/μL)</b>	0.6 ± 0.3	0.6 ± 0.3	1.1 ± 0.9	0.4 ~ 2
<b>Monocyte (10<sup>3</sup>/μL)</b>	0.2 ± 0.2	0.4 ± 0.1	0.4 ± 0.2	0 ~ 0.4
<b>Lymphocyte (10<sup>3</sup>/μL)</b>	3.6 ± 1.2	4.7 ± 3.2	3.0 ± 1.4	1.3 ~ 8.4
<b>Hematocrit (%)</b>	44.3 ± 3.6	42.8 ± 2.5	42.4 ± 2.8	32.8 ~ 48
<b>Hemoglobin (g/dL)</b>	15.7 ± 1.3	14.7 ± 1.1	14.5 ± 0.9	10 ~ 16.1
<b>Platelet (10<sup>6</sup>/μL)</b>	262 ± 344	273 ± 340	261 ± 331	250 ~ 1540

**Supplemental Table 4. Antibodies for Immunofluorescence Staining**

Antibody	Vendor	Catalog#	Source	Clone #	Dilution
ARNO (Cyth2)	Abcam	Ab2728	Mouse mAb	10A12	1:20
Clathrin Heavy Chain	Cell Signaling Technology	2410	Rabbit Polyclonal	n/a	1:100
GEP100 (IQSEC1)	Abcam	Ab122442	Rabbit Polyclonal	n/a	1:25
LAMP1	Cell Signaling Technology	9091	Rabbit mAb	D2D11	1:200
RAB 5	Cell Signaling Technology	3547	Rabbit mAb	C8B1	1:200
VEGFR2 (KDR)	R&D Systems	MAB3571	Mouse mAb	89115	1:200

**Supplemental Table 5. Antibodies for Western Blotting**

Antibody	Vendor	Catalog#	Source	Clone #
VEGFR2	Cell Signaling Technology	2479	Rabbit mAb	55B11
VEGFR2 pY951	Cell Signaling Technology	4991	Rabbit mAb	15D2
VEGFR2 pY1059	Cell Signaling Technology	3817	Rabbit mAb	D5A6
VEGFR2 pY1175	Cell Signaling Technology	2478	Rabbit mAb	19A10
VEGFR2 pY1212	Cell Signaling Technology	2477	Rabbit mAb	11A3
pPLC $\gamma$	Cell Signaling Technology	2821	Rabbit Ab	n/a
PLC $\gamma$	Cell Signaling Technology	5690	Rabbit mAb	D9H10
pp38	Cell Signaling Technology	9211	Rabbit Ab	n/a
p38	Cell Signaling Technology	9212	Rabbit Ab	n/a
pERK1/2	Cell Signaling Technology	4377	Rabbit mAb	197G2
ERK1/2	Cell Signaling Technology	9107	Mouse mAb	3A7
pMARCKS	Cell Signaling Technology	8722	Rabbit mAb	D13E4
MARCKs	Cell Signaling Technology	5607	Rabbit mAb	D88D11
GAPDH	Cell Signaling Technology	5174	Rabbit mAb	D16H11
ARF6	Cell Signaling Technology	5740	Rabbit mAb	D12G6
Neuropilin	Santa Cruz	Sc-7239	Goat polyclonal	n/a
$\alpha$ Tubulin	Cell Signaling Technology	3873	Mouse mAb	DM1A
$\alpha$ -actinin	Cell Signaling Technology	6487	Rabbit mAb	D6F6
Caspase-3	Cell Signaling Technology	9662	Rabbit Ab	n/a
Caveolin pY14	BD Transduction laboratories	611338	Mouse	n/a
Caveolin	Cell Signaling Technology	3238	Rabbit Ab	n/a
ARNO	Abnova	H00009266-MO2	Mouse mAb	n/a
GEP100	Sigma	HPA038144	Rabbit polyclonal	n/a
VEGFR2 (Sepharose beads)	Cell Signaling Technology	5168	Rabbit mAb	55B11

**Supplemental Table 6. Table for siRNA Sequence**

Target Gene Symbol	siRNA ID	Sense Sequence 5' -> 3'	Vendor
Control	AllStars Neg. Control siRNA	Proprietary	QIAGEN
<i>ARF6</i>	SI02757286	CAACGTGGAGACGGTGACTTA	QIAGEN
<i>GEP100</i>	SI03019408	CTGAAGGGTAGCAGTAATGAA	QIAGEN
<i>ARNO</i>	SI00061299	CGCUGUUGGUAAUCUUAUUTT	QIAGEN

**Supplemental Table 7. Table for Angiographic Sign Scores**

<b>Angiographic Sign</b>	<b>Scores</b>
Optic Disc Hyperfluorescence	
Normal staining of the scleral rim	0
Staining of the disc with distinct margins:	
Partial	1
Diffuse	2
<hr/>	
<i>Pinpoint leaks</i>	
Limited area or at one focus	1
Extensive	2
<hr/>	
<i>Neovascularization</i>	
No hyperfluorescence	I
Hyperfluorescence without leakage	II
Hyperfluorescence early to mid-transit with leakage	III
Hyperfluorescence early or mid-transit with leakage extending beyond the borders of the treated area	IV