Supplemental Material for

Targeted deletion of *Vegfa* in adult induces vision loss

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Supplemental Methods

Table S1-2

Figure S1-4

Supplemental Methods

Animals

Transgenic mice carrying the human vitelliform macular dystrophy-2 (*VMD2*) promoter-directed reverse tetracycline-dependent transactivator (rtTA) and the tetracycline-responsive element (TRE)-directed Cre recombinase (*VMD2-Cre* mice) (13) (provided by Dr. Y.Z. Le of University of Oklahoma Health. Sciences Center, Oklahoma City, USA) were mated with $Vegfa^{flox/flox}$ mice (29), $Hif1a^{flox/flox}$ mice (30), or $Epas1^{flox/flox}$ mice (31) (provided by Dr. R.S. Johnson of University of Cambridge, Cambridge, UK). Control littermates harboring floxed alleles but no Cre-recombinase were utilized after confirming phenotypic effects of doxycycline or Cre recombination activity in RPE (Fig. S1). To induce gene deletion, 80 μg/g body weight of doxycycline was injected into six to eight week-old adult transgenic mice intraperitoneally once a day for three days (counting began after the day of the first injection).

Electron microscopy analysis

Eye cups containing the retina and choroid from each mouse were fixed in 4% paraformaldehyde plus 1.5% glutaraldehyde in 0.1 M cacodylate buffer overnight at

4°C followed by rinsing in 0.1 M Na cacodylate buffer for 1 hour. The eye cups were then postfixed in 1% OsO_4 in 0.1 M cacodylate buffer for 2 hours, followed by another 1-hour wash and dehydration with graded ethanol solutions. Samples were incubated overnight in a 1:2 mixture of propylene oxide and Epon/Araldite (Sigma-Aldrich) and then placed in 100% resin followed by embedding. The blocks were sectioned and used for high-magnification electron microscopy analysis.

Ganzfeld ERG

After overnight dark adaptation, mice were anesthetized under dim red light by intraperitoneal injection of 15 mg/kg ketamine and 7 mg/kg xylazine. Full-field ERGs were recorded from the corneal surface of each eye after pupil dilation (1% tropicamide and 2.5% phenylephrine) using active contact lens electrodes (Mayo) placed on the cornea together with a mouth reference and tail ground electrode. A computerized system with an electronically controlled Ganzfeld dome was used (Espion E2 with Colordome; Diagnosys). In the dark-adapted state (scotopic), we recorded rod and mixed cone/rod responses to a series of white flashes of increasing intensities (1×10^{-5} to 50 $cd{\bullet}s/m^2$). In the light-adapted state (photopic), with a 30 cd/m^2 background, cone responses to 1-Hz (0.63 to 20 $cd{\bullet}s/m^2$) and 30-Hz (3.98, 10, and 20 $cd{\bullet}s/m^2$) flicker stimuli were recorded. All ERG responses were filtered at 0.3–500 Hz, and signal averaging was applied.

Immunohistochemistry

Retinas or RPE/choroid complexes were dissected and prepared for whole mounts or sectioning. For preparation of retinal cross-sections, dissected retinas were laid flat with 4 radial relaxing incisions, placed in 4% PFA, and incubated at 4°C overnight. Retinas were then placed in 20% sucrose at 4°C for 4 hours and embedded in Tissue-Tek OCT compound (Sakura FineTechnical) for cryosectioning. Antibodies were purchased from Life Technologies and included red/green opsin (1:200), and rhodopsin (1:500). For RPE/choroid whole mount staining, fluorescent-conjugated isolectin Griffonia Simplicifolia IB-4 was used (GS-lectin; Life Technologies). Images were obtained using a confocal microscopy (LSM710, Carl Zeiss)

In vivo imaging

Indocyanine green angiography and optical coherence tomography were performed using Spectralis (Heidelberg Engineering) as we previously described (33). Mice were anesthetized by an i.p. injection of 15 mg/kg ketamine and 7 mg/kg xylazine, and ICG

(50 µg/g body weight) was injected intraperitoneally. Pupils were dilated immediately before imaging with 1% tropicamide and 2.5% phenylephrine.

Transcriptomic arrays

For mRNA arrays, total RNA was prepared from RPE/choroid complexes using the RNeasy Plus Mini kit (Qiagen) and was reverse transcribed using RT² First Strand cDNA Kit (Qiagen). mRNA PCR arrays for hypoxia signaling, glucose metabolism, and fatty acid metabolism (Mouse Angiogenesis RT² Profiler™ PCR Array, PAMM-024, Qiagen) were performed according to the manufacturer's instructions. Quantitative PCR assays were performed on a real-time PCR System (ABI 7900HT Fast, Life Technologies).

Laser-induced CNV

Laser photocoagulation was performed at four spots per eye around the optic disk with the wavelength of 532 nm, the power of 200 mW, the duration of 100 ms, and the spot size of 75 µm, using a slit-lamp laser photocoagulation system (Novus Spectra; Lumenis) as previously described (34). One week after laser injury, mice were sacrificed and RPE/choroid whole mounts were prepared for CNV volume quantification. For mRNA array analysis, RPE/choroid complexes were obtained 3 days after laser irradiation. Horizontal optical sections of CNV were obtained every 1 μm step from the surface to the deepest focal plane. The area of CNV was measured by ImageJ (National Institutes of Health) and accumulated to obtain the volume.

Statistical analysis

Comparisons between the mean variables of two groups were performed by a two-tailed Student's t-test for mRNA arrays (RT^2 Profiler PCR Array Data Analysis Template v3.3; Qiagen), or others (Excel; Microsoft). *P* value less than 0.05 was considered significant.

Study approval

All procedures involving animals were approved by the Scripps Research Institute Animal Care Committee that ensures all federal animal experimentation guidelines are met.

Table S1. Complete data sets from qPCR gene-profiling analyses of 84 angiogenesis-related genes in naïve *Vegfa*, *Hif1a*, *Epas1*, and *Hif1a*;*Epas1* mutant RPE/Choroids 3 days post-induction.

Symbol	**Description**	***Vegfa***		***Hif1a***		***Epas1***		***Hif1a;Epas1***	
		Fold change	**p-value**	**Fold change**	**p-value**	**Fold change**	**p-value**	**Fold change**	**p-value**
Angpt1	Angiopoietin 1	-1.34	0.413585	1.44	0.26444	1.89	0.009391	1.03	0.753598
Angpt2	Angiopoietin 2	1.3	0.141577	1.69	0.153693	1.5	0.272844	1.04	0.953264
Anpep	Alanyl (membrane) aminopeptidase	-1.41	0.003938	-1.75	0.111973	-1.26	0.373745	1.28	0.375612
Bai1	Brain-specific angiogenesis inhibitor 1	1.47	0.223936	1.18	0.61102	1.57	0.306897	1.52	0.540079
Ccl11	Chemokine (C-C motif) ligand 11	1.65	0.021573	1.49	0.206098	1.93	0.001735	-1.31	0.307342
Ccl2	Chemokine (C-C motif) ligand 2	-1.96	0.221456	1.03	0.935642	1.25	0.475604	-1.1	0.648763
Cdh5	Cadherin 5	-1.53	0.214256	-1.14	0.521722	1.16	0.413375	1.12	0.48664
Col18a1	Collagen, type XVIII, alpha 1	-1.73	0.000268	-1.23	0.422148	1.49	0.302076	1.23	0.584866
Col4a3	Collagen, type IV, alpha 3	-1.18	0.543812	1.12	0.764706	1.42	0.403871	1.25	0.119333

Csf3	Colony stimulating factor 3 (granulocyte)	1.1	0.281964	-1.05	0.921171	1.18	0.763564	1.26	0.317169
Ctgf	Connective tissue growth factor	1.04	0.992852	1.22	0.201868	1.06	0.672108	-1.24	0.660256
Cxcl1	Chemokine (C-X-C motif) ligand 1	-1.76	0.008988	1.39	0.193077	1.33	0.20067	-1.03	0.932115
Cxcl2	Chemokine (C-X-C motif) ligand 2	2.8	0.05201	2.02	0.176471	1.54	0.297605	1.59	0.281366
Cxcl5	Chemokine (C-X-C motif) ligand 5	1.22	0.7213	1.52	0.224189	1.87	0.010285	-1.21	0.462104
Tymp	Thymidine phosphorylase	-3.8	0.001734	2.05	0.341018	2.69	0.179331	-2.27	0.663608
S1pr1	Sphingosine-1-phosphate receptor 1	-1.12	0.635685	1.03	0.855271	1.07	0.652293	1.24	0.120569
Efna1	Ephrin A1	-1.41	0.178414	-1.41	0.121185	-1.08	0.615045	1.11	0.548721
Efnb2	Ephrin B2	-1.33	0.023749	1.15	0.371299	1.09	0.571925	1.14	0.488908
Egf	Epidermal growth factor	-1.12	0.766746	-1.73	0.227011	-1.49	0.28664	-1.24	0.42069
Eng	Endoglin	-1.38	0.252153	-1.68	0.153268	-1.22	0.406361	1.26	0.356316
Epas1	Endothelial PAS domain protein	-1.1	0.21504	-2.25	0.076373	-1.83	0.023472	-2.97	0.086782

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Ephb4	Eph receptor B4	-1.28	0.243319	-1.28	0.274985	-1.01	0.894101	1.21	0.265727
Ereg	Epiregulin	-1.46	0.002396	-1.4	0.526383	-1.52	0.280077	1.52	0.268226
F2	Coagulation factor II	-1.82	0.124291	1.66	0.261644	1.35	0.364178	-1.11	0.949614
Fgf1	Fibroblast growth factor 1	-1.11	0.521759	1	0.972462	1.26	0.151424	-1.25	0.149164
Fgf2	Fibroblast growth factor 2	1.23	0.019132	1.01	0.941347	1.29	0.310001	-1.16	0.421374
Fgf6	Fibroblast growth factor 6	-1.29	0.665944	1.23	0.408293	-1.13	0.725849	-1.27	0.798363
Fgfr3	Fibroblast growth factor receptor 3	-1.22	0.002325	-1.6	0.257141	-1.39	0.11319	-1.14	0.416329
Figf	C-fos induced growth factor	-1.44	0.142495	1.72	0.096738	1.44	0.290538	-1.34	0.672405
Flt1	FMS-like tyrosine kinase 1	-1.08	0.032614	1.21	0.460796	1.29	0.314362	1.02	0.996145
Fzd5	Frizzled homolog 5 (Drosophila)	1.16	0.020965	1.87	0.138911	1.35	0.219182	-1.23	0.647013
Gna13	Guanine nucleotide binding protein, alpha 13	-1.46	0.094009	1.34	0.146115	1.21	0.283406	1	0.989866
Hand2	Heart and neural crest derivatives expressed transcript 2	-3.47	0.001427	1.81	0.220895	1.88	0.181627	-1.97	0.523564

Hgf	Hepatocyte growth factor	-1.85	0.026597	1.19	0.449565	1.59	0.015265	-1.03	0.457424
Hif1a	Hypoxia inducible factor 1, alpha subunit	-1.05	0.684424	-2.48	0.042448	1.95	0.065454	-2.75	0.064844
Ifng	Interferon gamma	-2.49	0.139935	1.35	0.602779	-1.22	0.722856	1.1	0.914201
Igf1	Insulin-like growth factor 1	-1.92	0.00534	1.13	0.556216	1.41	0.080642	1.08	0.58172
Il1b	Interleukin 1 beta	1.39	0.195485	1.94	0.161591	2.15	0.068343	1.25	0.413902
Il6	Interleukin 6	-1.78	0.009261	2.46	0.266808	1.85	0.162402	-2.45	0.176681
Itgav	Integrin alpha V	-1.78	0.000975	1.09	0.575465	1.26	0.053416	-1.12	0.298615
Itgb3	Integrin beta 3	-2.65	0.000119	-1.11	0.367033	1.2	0.299618	1.11	0.58626
Jag1	Jagged 1	1.17	0.109726	1.03	0.764804	1.08	0.34165	1.13	0.163439
Kdr	Kinase insert domain protein receptor	-1.98	0.149414	1.11	0.602043	1.27	0.219733	1.29	0.174536
Lama5	Laminin, alpha 5	-1.44	0.002422	-1.06	0.912131	1.34	0.167732	1.23	0.172055
Lect1	Leukocyte cell derived chemotaxin 1	1.23	0.180073	2.23	0.163337	1.95	0.258268	-1.18	0.906
Lep	Leptin	-1.37	0.212414	1.05	0.907086	-1.87	0.370516	-1.87	0.739498

Mapk14	Mitogen-activated protein kinase 14	-1.52	0.002186	-1.39	0.061261	-1.09	0.783305	1.22	0.486672
Mdk	Midkine	-1.36	0.00584	1.14	0.589354	1.36	0.173203	-1.19	0.533306
Mmp19	Matrix metallopeptidase 19	-1.09	0.525064	-1.18	0.352271	1.1	0.464103	1.18	0.344209
Mmp2	Matrix metallopeptidase 2	1.02	0.973444	1.09	0.258416	-1.21	0.473528	-1.04	0.954624
Mmp9	Matrix metallopeptidase 9	6.08	0.001287	1.35	0.118035	1.2	0.398476	1.24	0.161787
Npr1	Natriuretic peptide receptor 1	-1.48	0.076702	-1.72	0.054433	-1.25	0.444566	1.3	0.446015
Nrp1	Neuropilin 1	-1.51	0.067765	1.08	0.551169	1.08	0.542382	-1.08	0.761728
Nrp2	Neuropilin 2	-1.45	0.007964	-1.17	0.596789	1.39	0.351551	1.38	0.255685
Pdgfa	Platelet derived growth factor, alpha	-1.44	0.011454	-1.64	0.091396	-1.22	0.418402	-1.04	0.817247
Pecam1	Platelet/endothelial cell adhesion molecule 1	-1.39	0.331894	-1.2	0.245105	1.09	0.538363	1.08	0.598698
Pgf	Placental growth factor	-1.22	0.611459	1.01	0.958914	1.09	0.659936	-1.19	0.449321
Plau	Plasminogen activator, urokinase	-1.46	0.041819	-1.37	0.110929	1.09	0.673124	1.23	0.512246
Plg	Plasminogen	-1.78	0.009261	1.81	0.220895	1.88	0.181627	-1.97	0.523564

Plxdc1	Plexin domain containing 1	-1.93	0.067919	1.03	0.931484	1.38	0.277325	1.32	0.180729
Ptgs1	Prostaglandin-endoperoxide synthase 1	-1.02	0.991682	-1.1	0.393071	-1.08	0.4896	-1.09	0.534958
Serpinf1	Serine (or cysteine) peptidase inhibitor, clade F, member 1	-1.15	0.767346	1.22	0.393652	1.03	0.792538	-1.15	0.842248
Smad5	MAD homolog 5 (Drosophila)	1.11	0.27548	1.73	0.153989	1.34	0.406406	1.01	0.991583
Sphk1	Sphingosine kinase 1	1.82	0.000616	-1.55	0.063739	-1.2	0.298705	1.07	0.706168
Stab1	Stabilin 1	-1.33	0.315313	-1.54	0.076955	-1.18	0.387896	1.07	0.686033
Tbx1	T-box 1	-1.22	0.485835	1.17	0.477756	-1.08	0.988315	1.11	0.844596
Tbx4	T-box 4	-1.45	0.325003	-1.46	0.189404	-1.72	0.275038	1.78	0.21975
Tek	Endothelial-specific receptor tyrosine kinase	1.07	0.515444	1.53	0.172147	1.22	0.492206	-1.03	0.974651
Tgfa	Transforming growth factor alpha	-1.37	0.209927	-1.45	0.088639	-1.08	0.867723	-1.12	0.566362
Tgfb1	Transforming growth factor, beta 1	-1.95	0.118792	-1.29	0.300013	1.1	0.695248	1.48	0.181421
Tgfb2	Transforming growth factor, beta 2	-1.49	0.000042	-1.09	0.740676	1.56	0.212142	1	0.907187

Tgfb3	Transforming growth factor, beta 3	-1.25	0.719313	-1.33	0.166582	1.03	0.930625	1.26	0.305778
Tgfbr1	Transforming growth factor, beta receptor I	1.75	0.00853	-1.2	0.23823	-1.01	0.856686	1.08	0.475733
Thbs1	Thrombospondin 1	-1.78	0.000632	1.29	0.309703	1.54	0.110338	-1.15	0.51713
Thbs2	Thrombospondin 2	-1.3	0.316161	1.04	0.869902	-1	0.990211	1.27	0.33332
Timp1	Tissue inhibitor of metalloproteinase 1	-1.77	0.111267	1.12	0.81787	1.4	0.485571	1.15	0.454325
Timp2	Tissue inhibitor of metalloproteinase 2	-1.4	0.042988	-1.2	0.384877	1.17	0.41614	1.08	0.596163
Tmprss6	Transmembrane serine protease 6	-2.24	0.024009	1.2	0.432868	-1.17	0.343052	1.54	0.194879
Tnf	Tumor necrosis factor	-2.15	0.004883	-1.08	0.65297	1.19	0.484798	1.02	0.816678
Tnfaip2	Tumor necrosis factor, alpha-induced protein 2	-3.26	0.000447	-1.66	0.056905	1.06	0.699477	1.68	0.283888
Tnfsf12	Tumor necrosis factor (ligand) superfamily, member 12	-1.44	0.098118	-1.12	0.67804	1.11	0.797424	1.2	0.413639
Vegfa	Vascular endothelial growth	-1.41	0.047516	-1.09	0.716375	1.39	0.362673	1.09	0.739881

	factor A								
Vegfb	Vascular endothelial growth factor B	-1.83	0.000006	-1.45	0.080076	-1.12	0.759113	1.16	0.66229
Vegfc	Vascular endothelial growth factor C	-1.71	0.016196	1.64	0.164829	1.65	0.134874	-1.04	0.922215

Fold-change and p-values are listed. Note that 32 of 84 genes are significantly downregulated in *Vegf* mutants whereas few or no significantly dysregulated genes are observed in *Hif1a*, *Epas1*, or *Hif1a;Epas1* mutants. This table is associated with Figure 2F and Figure S2C.

Table S2. Complete data sets from qPCR gene-profiling analyses of 84 angiogenesis-related genes in laser-irradiated B6 wild-type, *Vegfa*, *Hif1a*, *Epas1*, and *Hif1a;Epas1* mutant RPE/Choroids 3 days post laser irradiation.

Symbol	**Description**	**Laser:B6**		**Laser:*Vegfa***		**Laser:*Hif1a***		**Laser:*Epas1***		**Laser:*Hif1a;Epas1***	
		Fold change	**p-value**	**Fold change**	**p-value**	**Fold change**	**p-value**	**Fold change**	**p-value**	**Fold change**	**p-value**
Angpt1	Angiopoietin 1	2.57	0.057941	-2.96	0.046532	1.92	0.006742	1.95	0.017546	1.66	0.080341
Angpt2	Angiopoietin 2	1.37	0.05142	-1.54	0.05124	1.72	0.139743	1.56	0.228228	1.96	0.033683
Anpep	Alanyl (membrane) aminopeptidase	1.01	0.912503	-1.34	0.146721	-1.58	0.270667	1.02	0.891484	-1.81	0.092354
Bai1	Brain-specific angiogenesis inhibitor 1	4.78	0.13157	1.14	0.658676	1.98	0.13333	2.39	0.17993	3.41	0.319957
Ccl11	Chemokine (C-C motif) ligand 11	1.61	0.234886	-2.74	0.101953	1.93	0.001806	1.47	0.189679	1.52	0.181263
Ccl2	Chemokine (C-C	-2.64	0.063335	3.52	0.00462	-1.05	0.725322	1.14	0.635614	-1.18	0.399753

	motif) ligand 2										
Cdh5	Cadherin 5	1.17	0.37307	-1.11	0.915541	1.01	0.959844	1.29	0.301618	-1.1	0.617451
Col18a1	Collagen, type XVIII, alpha 1	1.17	0.602362	1.2	0.585704	1.24	0.537785	1.84	0.140498	-1.11	0.596432
Col4a3	Collagen, type IV, alpha 3	2.31	0.083459	-2.82	0.041117	1.52	0.273024	1.77	0.105407	1.67	0.16291
Csf3	Colony stimulating factor 3 (granulocyte)	3.99	0.097507	-1.38	0.514474	1.15	0.816387	1.49	0.285888	1.45	0.349808
Ctgf	Connective tissue growth factor	1.34	0.069779	-1.89	0.027443	1.08	0.618947	-1.18	0.826803	1.21	0.250068
Cxcl1	Chemokine (C-X-C motif) ligand 1	1.24	0.241744	-1.39	0.17266	1.51	0.063183	1.29	0.257639	1.49	0.075625
Cxcl2	Chemokine	2.1	0.02792	1.12	0.584274	2.18	0.138152	2.46	0.004525	2.61	0.04226

	(C-X-C motif) ligand 2										
Cxcl5	Chemokine (C-X-C motif) ligand 5	2.13	0.050167	2.18	0.082495	1.93	0.010344	1.54	0.148668	1.69	0.104002
Tymp	Thymidine phosphorylase	1.65	0.263812	-2.8	0.199849	2.92	0.156751	1.18	0.502378	2.42	0.256395
S1pr1	Sphingosine-1-phosphate receptor 1	1.15	0.291132	1.15	0.349519	1.15	0.245082	1.32	0.096291	1.23	0.161164
Efna1	Ephrin A1	1.44	0.082963	-1.76	0.03905	-1.29	0.329561	1.03	0.877287	-1.31	0.278728
Efnb2	Ephrin B2	1.62	0.018249	-1.76	0.039774	1.12	0.451908	1.25	0.097188	1.28	0.162856
Egf	Epidermal growth factor	1.37	0.221431	-1.94	0.01368	-1.73	0.227785	-1.85	0.083132	-1.78	0.181653
Eng	Endoglin	1	0.902595	-1.23	0.470544	-1.53	0.250117	1.04	0.910484	-1.62	0.177374
Epas1	Endothelial PAS domain protein 1	2.06	0.022265	-2.3	0.013643	-1.92	0.439279	-1.08	0.864998	-2.5	0.016982

Ephb4	Eph receptor B4	1.29	0.121482	-1.38	0.128263	-1.14	0.622658	1.2	0.358955	-1.17	0.500847
Ereg	Epiregulin	3	0.028354	-3.26	0.211804	-2.03	0.181703	-1	0.957754	-1.49	0.379243
F2	Coagulation factor II	1.49	0.280163	-1.5	0.321263	2.05	0.103782	1.22	0.489206	2.94	0.021281
Fgf1	Fibroblast growth factor 1	1.38	0.028139	-2.14	0.002063	1.21	0.255498	1.01	0.945855	1.11	0.264553
Fgf2	Fibroblast growth factor 2	1.5	0.024565	1.59	0.378702	1.33	0.253699	1.11	0.533719	1.41	0.267783
Fgf6	Fibroblast growth factor 6	1.04	0.804206	-1.42	0.2721	1.22	0.430693	-1.43	0.594782	1.32	0.252486
Fgfr3	Fibroblast growth factor receptor 3	2.46	0.00103	-6.44	0.0002	-1.82	0.048641	-1.58	0.047745	-1.97	0.0229
Figf	C-fos induced growth factor	1.46	0.175267	-2.45	0.04448	1.74	0.085426	1.07	0.715401	1.37	0.355226
Flt1	FMS-like tyrosine kinase 1	1.85	0.01781	-1.63	0.031457	1.57	0.028948	1.31	0.208924	1.7	0.033709

Fzd5	Frizzled homolog 5 (Drosophila)	1.98	0.033488	-1.5	0.19988	1.83	0.160578	1.1	0.646884	2.36	0.016301
Gna13	Guanine nucleotide binding protein, alpha 13	1.12	0.128566	-1.18	0.187415	1.37	0.102802	1.21	0.285464	1.45	0.020269
Hand2	Heart and neural crest derivatives expressed transcript 2	1.51	0.288951	-2.27	0.038986	2.24	0.051986	-1.05	0.764433	1.77	0.242934
Hgf	Hepatocyte growth factor	1.53	0.037423	-1.12	0.461606	1.51	0.029126	1.54	0.017156	1.25	0.300628
Hif1a	Hypoxia inducible factor 1, alpha subunit	1.91	0.024459	-1.28	0.393287	1.03	0.852512	1.79	0.13696	1.03	0.866506
Ifng	Interferon gamma	3.56	0.115383	-1.82	0.401328	-1.22	0.73003	-1.11	0.792213	-1.04	0.815723

Igf1	Insulin-like growth factor 1	1.11	0.534525	-1.06	0.775955	1.41	0.077788	1.51	0.034317	1.12	0.596252
Il1b	Interleukin 1 beta	1.29	0.259766	2.12	0.010205	2.27	0.05898	2.68	0.003409	2.44	0.024125
Il6	Interleukin 6	3.04	0.084168	3.44	0.003022	5.76	0.025789	3.02	0.257088	4.44	0.086696
Itgav	Integrin alpha V	1.29	0.103654	-1.78	0.008177	1.23	0.089362	1.12	0.404731	1.1	0.523141
Itgb3	Integrin beta 3	-1.42	0.081734	2.07	0.062591	1.04	0.806912	1.33	0.209792	-1.1	0.390195
Jag1	Jagged 1	1.79	0.010227	-1.61	0.015978	1.09	0.310078	1.22	0.076253	1.22	0.168749
Kdr	Kinase insert domain protein receptor	2.22	0.00659	-1.56	0.265214	1.38	0.064886	1.65	0.05184	1.71	0.190047
Lama5	Laminin, alpha 5	1.89	0.06323	-1.73	0.083275	1.19	0.468758	1.65	0.021394	1.24	0.41487
Lect1	Leukocyte cell derived chemotaxin 1	2.09	0.015802	1.41	0.391283	2.51	0.101613	1.66	0.36051	3.26	0.010073
Lep	Leptin	-2.11	0.222252	-2.94	0.038311	-1.57	0.416211	-3.5	0.293085	-1.56	0.419556
Mapk1	Mitogen-activate	3.67	0.347047	-3.31	0.35245	-1.19	0.624873	1.12	0.550152	-1.22	0.477269

4	d protein kinase 14										
Mdk	Midkine	1.09	0.665321	-1.74	0.050924	1.4	0.116013	1.14	0.594596	1.09	0.672684
Mmp19	Matrix metallopeptidase 19	-1.43	0.053628	2.1	0.014539	-1.05	0.918663	1.3	0.188734	-1.18	0.340211
Mmp2	Matrix metallopeptidase 2	-1.38	0.28124	1.1	0.783382	-1.08	0.802783	-1.27	0.486299	-1.1	0.767007
Mmp9	Matrix metallopeptidase 9	-1.99	0.048716	4.1	0.000325	1.32	0.166741	1.48	0.066224	1.29	0.211685
Npr1	Natriuretic peptide receptor 1	1.57	0.024154	-1.99	0.006672	-1.56	0.256495	1.04	0.686993	-1.75	0.041399
Nrp1	Neuropilin 1	1.15	0.085876	-1.72	0.012036	1.1	0.44379	1	0.901313	1.04	0.742057
Nrp2	Neuropilin 2	1.41	0.314607	-1.03	0.972071	1.21	0.642517	1.91	0.13872	1.12	0.844816

Pdgfa	Platelet derived growth factor, alpha	1.17	0.577385	-1.76	0.064186	-1.41	0.29322	-1.26	0.313953	-1.59	0.107665
Pecam1	Platelet/endothelial cell adhesion molecule 1	1.13	0.37447	-1.01	0.904282	-1.05	0.840281	1.18	0.382615	-1.15	0.364842
Pgf	Placental growth factor	2.34	0.001013	-3.27	0.00002	1.11	0.579278	-1.09	0.738939	1.07	0.761351
Plau	Plasminogen activator, urokinase	-1.16	0.440409	1.19	0.275189	-1.11	0.881508	1.34	0.33306	-1.43	0.07604
Plg	Plasminogen	1.63	0.190497	-1.46	0.425618	2.24	0.051986	-1.05	0.764433	1.77	0.242934
Plxdc1	Plexin domain containing 1	1.74	0.064956	-1.67	0.011138	1.35	0.331619	1.82	0.088922	1.34	0.338324
Ptgs1	Prostaglandin-endoperoxide synthase 1	-1.01	0.988621	-1.16	0.639899	-1.17	0.198172	-1.17	0.273266	-1.23	0.170454

Serpinf1	Serine (or cysteine) peptidase inhibitor, clade F, member 1	1.21	0.432409	-1.61	0.244268	1.17	0.481661	-1.11	0.983738	1.29	0.257899
Smad5	MAD homolog 5 (Drosophila)	1.13	0.304517	-1.31	0.112165	1.75	0.146554	1.35	0.396208	2.08	0.021344
Sphk1	Sphingosine kinase 1	-1.6	0.025418	2.11	0.004773	-1.47	0.159925	-1.12	0.585103	-1.25	0.795537
Stab1	Stabilin 1	-1.87	0.023241	1.65	0.007652	-1.41	0.212775	-1.1	0.686066	-1.5	0.105999
Tbx1	T-box 1	1.45	0.138808	-2.77	0.019578	1.15	0.509278	1.03	0.796658	1.28	0.203823
Tbx4	T-box 4	-1.27	0.216735	-1.33	0.359745	-1.38	0.279126	1.04	0.906345	-1.45	0.196282
Tek	Endothelial-specific receptor tyrosine kinase	1.3	0.174357	-1.76	0.057893	1.51	0.188427	1.18	0.529869	1.65	0.085987
Tgfa	Transforming growth factor	1.22	0.359958	-1.73	0.038839	-1.18	0.731784	-1.2	0.240011	-1.54	0.060224

	alpha										
Tgfb1	Transforming growth factor, beta 1	-1.24	0.282994	1.79	0.172222	-1.13	0.647655	1.63	0.167413	-1.1	0.771215
Tgfb2	Transforming growth factor, beta 2	1.77	0.097776	-1.11	0.530064	1.38	0.390542	1.56	0.10157	1.29	0.427717
Tgfb3	Transforming growth factor, beta 3	2.19	0.00564	-1.35	0.130105	-1.17	0.490896	1.3	0.322933	-1.17	0.490401
Tgfbr1	Transforming growth factor, beta receptor I	-1.03	0.743069	-1.2	0.203423	-1.14	0.463365	1.07	0.606902	-1.2	0.253441
Thbs1	Thrombospondin 1	1.42	0.048525	-1.32	0.117466	1.41	0.130259	1.34	0.189948	1.48	0.058028
Thbs2	Thrombospondin 2	-1.25	0.28693	1.63	0.155006	1.03	0.907846	1.26	0.311824	1.1	0.687203

Timp1	Tissue inhibitor of metalloproteinase 1	1.07	0.969056	1.58	0.209083	1.37	0.524299	1.61	0.260269	1.92	0.202499
Timp2	Tissue inhibitor of metalloproteinase 2	1.43	0.052325	-1.45	0.016679	1.01	0.939825	1.25	0.300803	-1.09	0.63906
Tmprss6	Transmembrane serine protease 6	2.01	0.011828	-1.92	0.181311	1.02	0.774773	1.32	0.302661	1.63	0.236838
Tnf	Tumor necrosis factor	4.62	0.021121	-3.35	0.03585	1.09	0.767532	1.21	0.425631	1.13	0.652939
Tnfaip2	Tumor necrosis factor, alpha-induced protein 2	1.54	0.054948	-1.63	0.022261	-1.29	0.77753	1.79	0.221039	-1.67	0.049895
Tnfsf12	Tumor necrosis factor (ligand)	1.34	0.427051	-1.04	0.880147	1.17	0.642445	1.34	0.376083	1.36	0.422035

	superfamily, member 12										
Vegfa	Vascular endothelial growth factor A	2.63	0.163294	-2.73	0.144783	1.31	0.474909	1.51	0.236882	1.23	0.586296
Vegfb	Vascular endothelial growth factor B	1.27	0.394393	-1.62	0.163299	-1.19	0.667822	1.03	0.831695	-1.32	0.236643
Vegfc	Vascular endothelial growth factor C	1.55	0.008534	-1.77	0.02666	1.82	0.066918	1.57	0.183306	1.9	0.034799

Fold-change and p-values are listed. Note that large number (22/84) of downregulated genes are observed in *Vegfa* mutants, whereas few genes are found in *Hif1a* or *Epas1* mutants. This table is associated with Figure 3C.

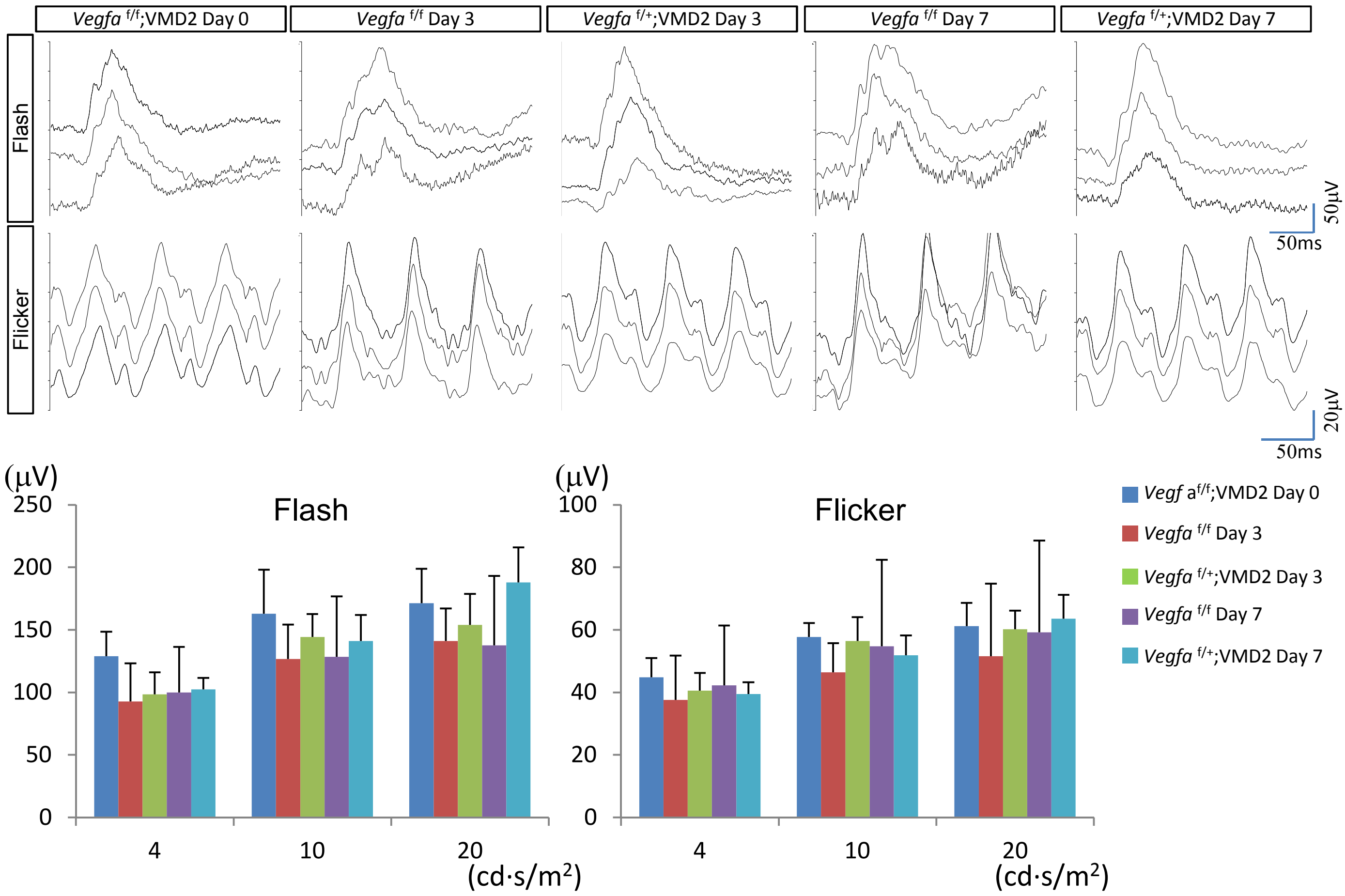

Figure S1 Photopic light-adapted ERG for multiple controls.

Note that *Vegfa*$^{f/f}$;(Cre(-)) or *Vegfa*$^{f/+}$;VMD2-Cre mice show no statistically significant differences in photopic ERG after 3 days or 7 days after doxycycline injection compared to *Vegf*$^{f/f}$;VMD2-Cre without doxycycline injection. These observations indicate that neither doxycycline injection nor Cre expression in RPE affect retinal function. n=4, respectively. Error bars indicate mean ± s.d.

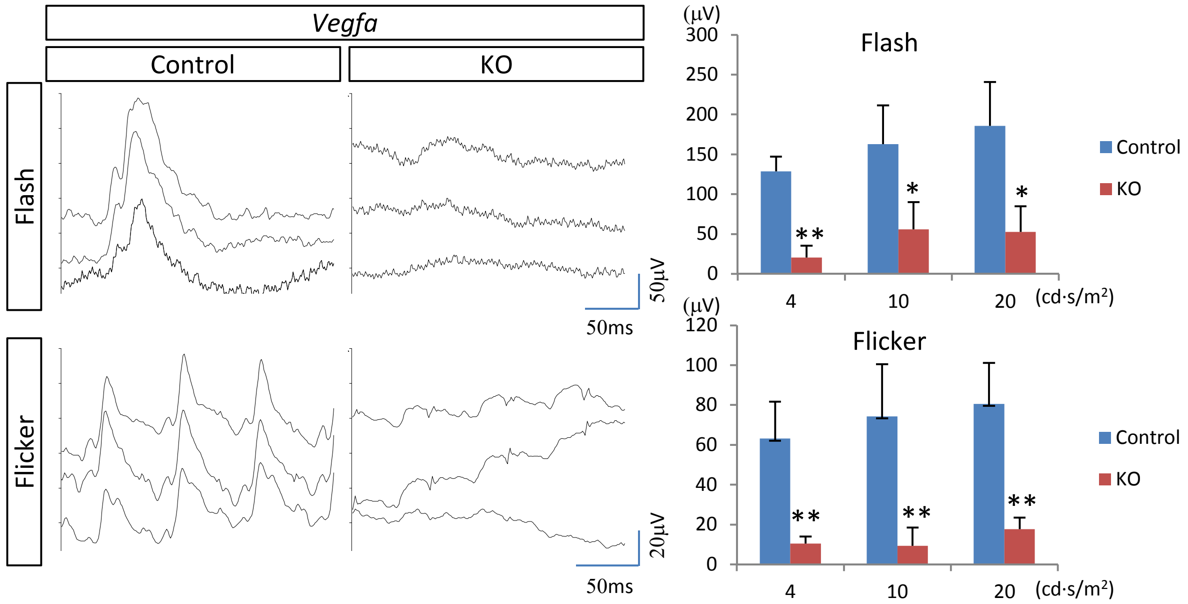

Figure S2 Photopic light-adapted ERG of *Vegfa* mutants at late stage (7 months after gene deletion). Note that significant dysfunction compared to age-matched control was observed. n=4, respectively. *$P<0.05$, **$P<0.01$; two-tailed Student's t tests. Error bars indicate mean ± s.d.

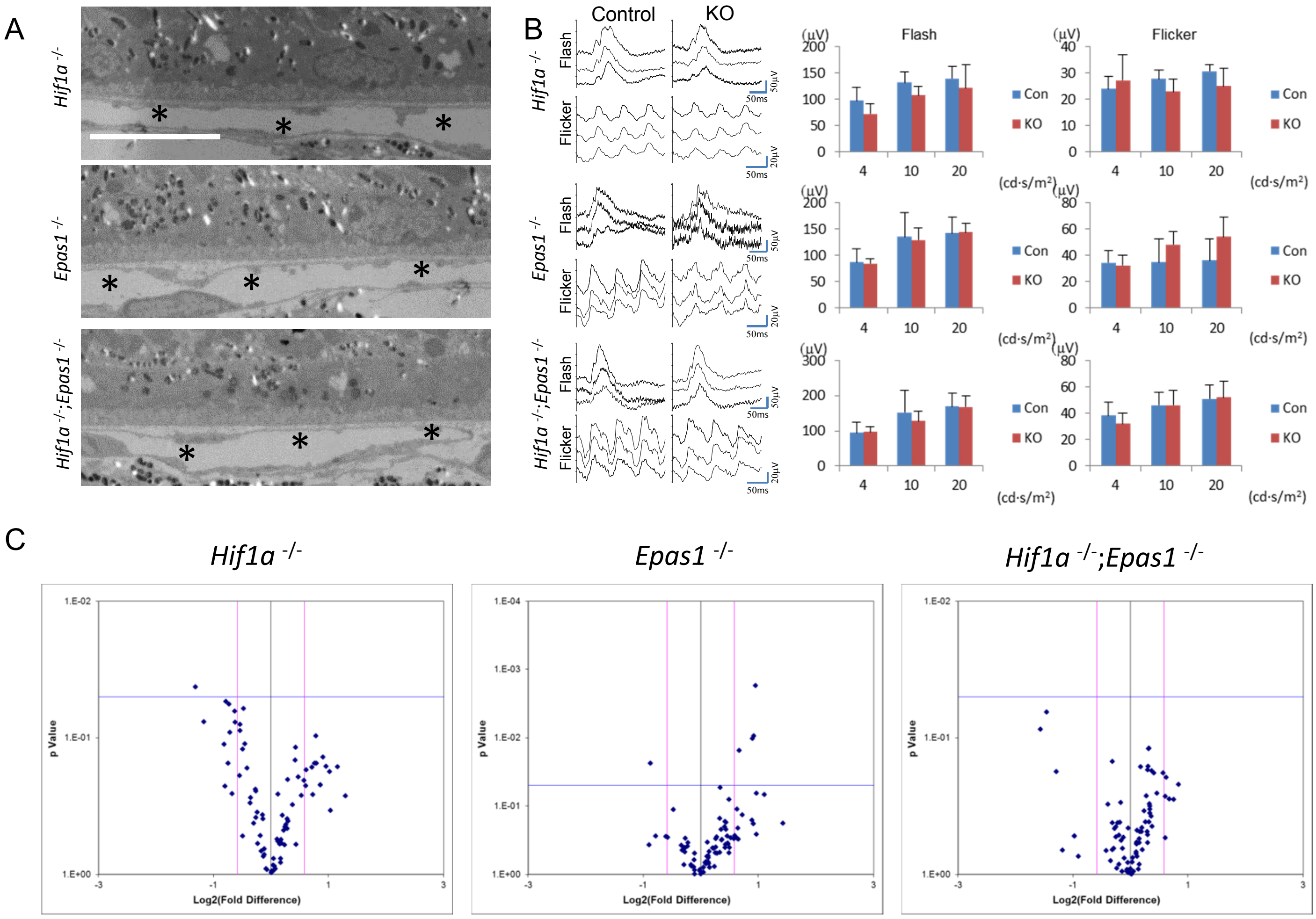

Figure S3 Phenotypes in naïve state HIF mutants.

(A) Electron micrographs for *Hif1a*, *Epas1*, and *Hif1a;Epas1* mutants 3 days post-induction. Note that the choriocapillaris in all these animals is intact (asterisks). (B) Photopic light-adapted ERG for *Hif1a*, *Epas1*, and *Hif1a;Epas1* mutants captured 7 days post-induction. Note that no statistical significant reduction of amplitudes is observed (n=4). (C) mRNA array for angiogenic genes in *Hif1a*, *Epas1*, and *Hif1a;Epas1* mutant RPE/choroids compared to controls in naïve states (n=3, respectively). Note that few or no significantly dysregulated genes are observed in naïve state *Hif1a*, *Epas1*, and *Hif1a;Epas1* mutants. Error bars indicate mean ± s.d. Scale bar: 10μm for A

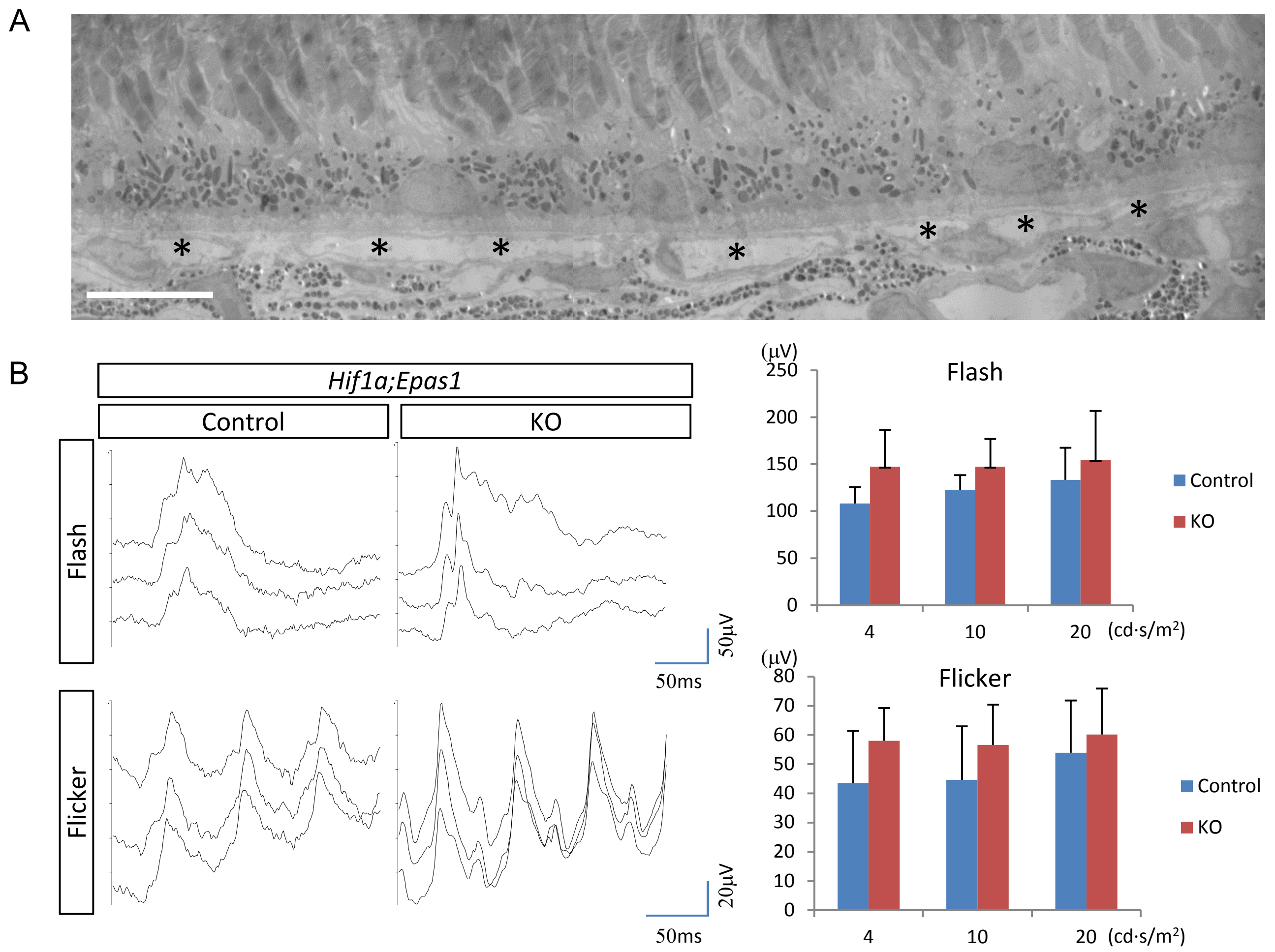

Figure S4 Phenotypes of *Hif1a/Epas1* double mutants at late stage (7 months after gene deletion). (A) Electron micrographs for *Hif1a;Epas1* mutants 7 months post-induction. Asterisks indicate intact choriocapillaris. (B) Photopic light-adapted ERG for *Hif1a;Epas1* mutants captured 7 months post-induction. Note that no significant functional and morphological change compared to age-matched control was observed. n=4, respectively. Error bars indicate mean ± s.d. Scale bar: 10μm for A