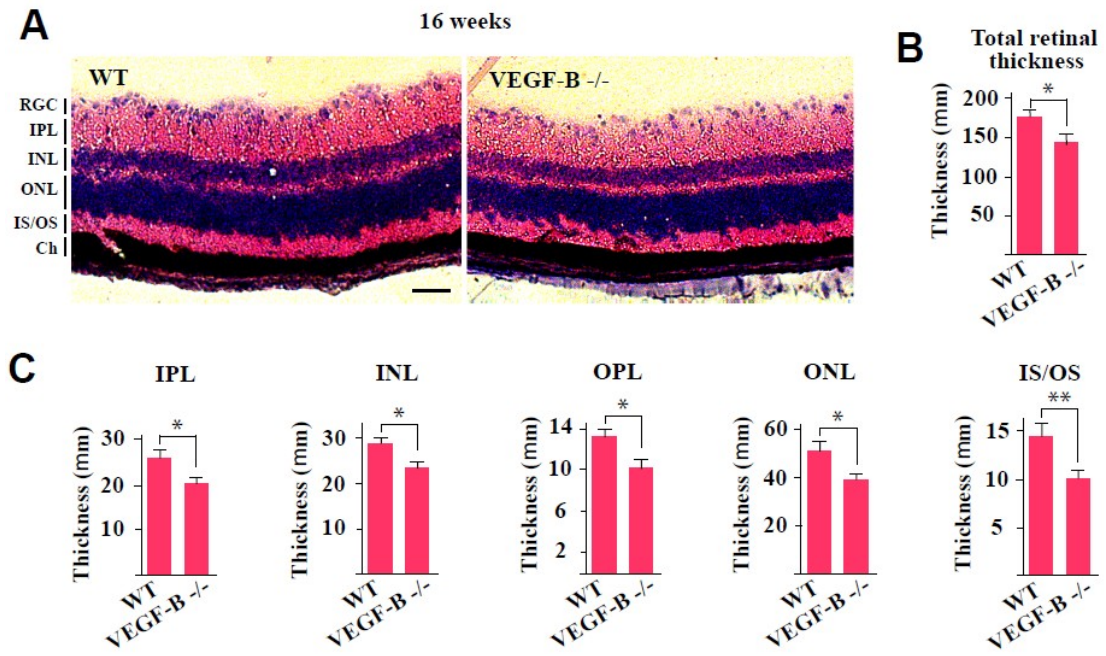

Fig.S1. Genetic deletion of *Vegf-b* leads to retinal degeneration in 16 week-old *Vegf-b*$^{-/-}$ mice

(**A-C**) H&E staining shows that the thickness of the retinal layers of 16 week-old *Vegf-b*$^{-/-}$ mice was significantly reduced (n = 8, $P< 0.05$), including the retinal ganglion cell layer (RGC), choroid, inner segment/outer segment (IS/OS), outer plexiform layer (OPL), outer nuclear layer (ONL), inner nuclear layer (INL) and inner plexiform layer (IPL, n = 8, $P< 0.01$ or 0.05).Scale bar: 50 μm

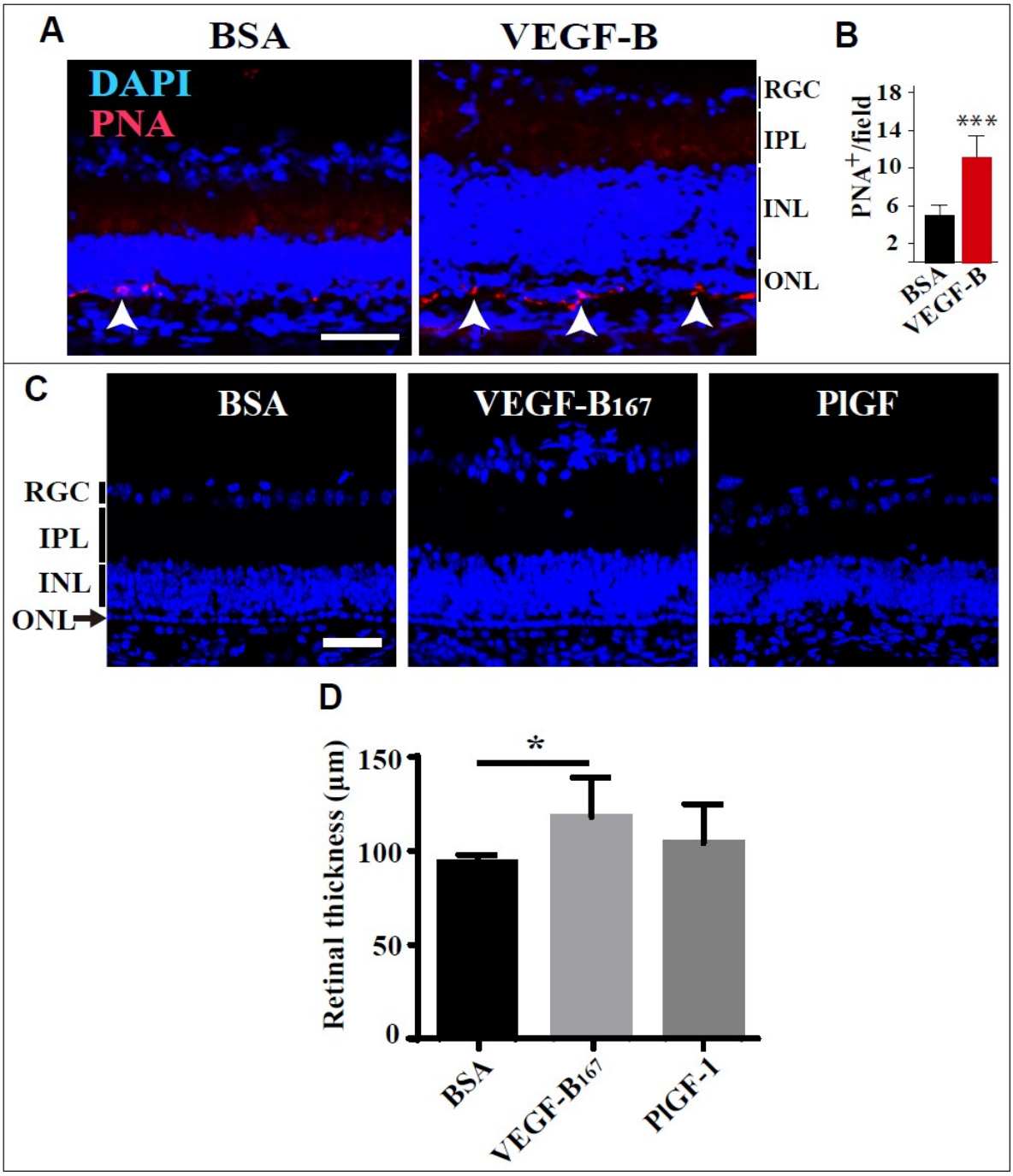

Fig. S2.VEGF-B treatment rescues retinal degeneration in rd1 mice

(**A, B**) Peanut agglutinin (PNA) staining reveals that in rd1 mice, intrvitreal injection of VEGF-B at P11 significantly resulted in more PNA^+ cones after VEGF-B treatment (n = 8, P < 0.001, arrow heads). (**C, D**) VEGF-B treatment (250 ng/eye) increased retinal thickness in rd1 mice (n = 6, *P*< 0.05). However,PlGF(250 ng/eye) did not show such an effect.Scale bars: 50 μm

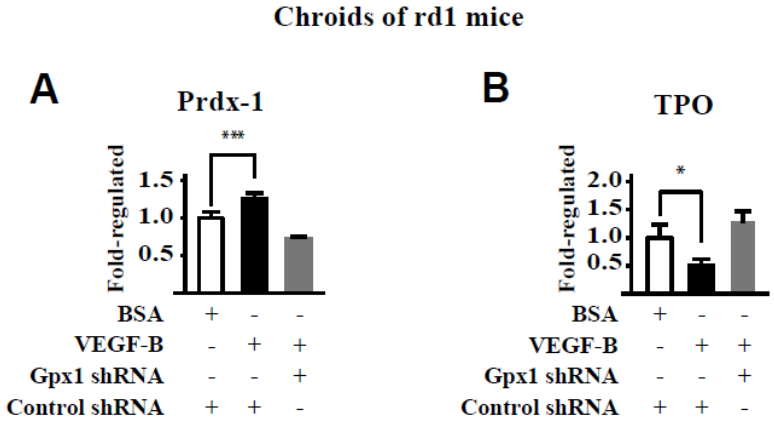

Fig. S3. Gpx1 is required for the regulatory effect of VEGF-B.

(**A**) In the choroids of the rd1 mice,real-time PCR shows that Gpx1 knockdown diminished the upregulatory effect of VEGF-B on the expression Prdx5. (**B**) In the choroids of the rd1 mice,real-time PCR shows that Gpx1 knockdown diminished the inhibitory effect of VEGF-B on the expression TPO (n = 8, $P< 0.001$ or 0.05).

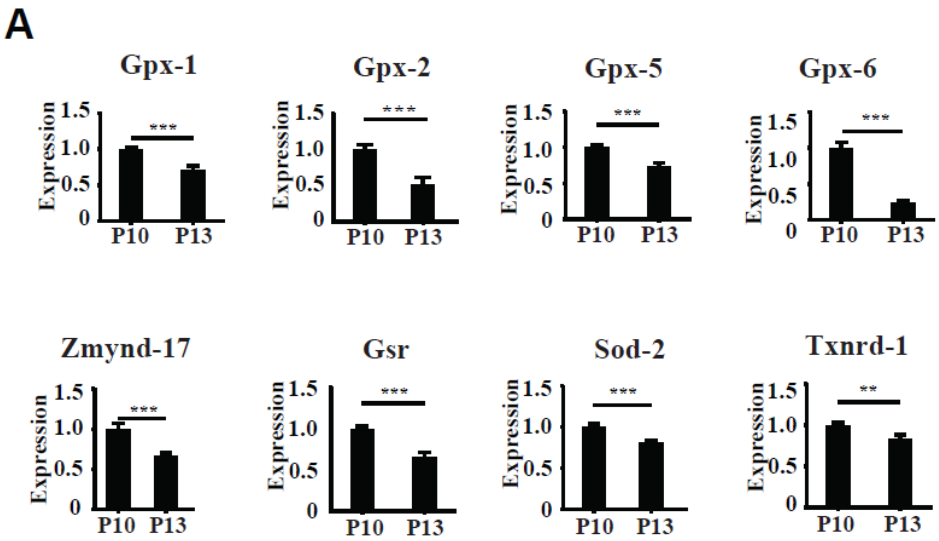

Fig. S4. Decreased expression of Gpx1 and other anti-oxidative genes in choroids of rd1 mice

(**A**) Upon retinal degeneration after postnatal day 10 (P10) in rd1 mice, Gpx1 expression level decreased together with those of some other anti-oxidative genes in the choroids of rd1 mice as shown by real-time PCR (n = 8, $P<$ 0.001 or 0.01).

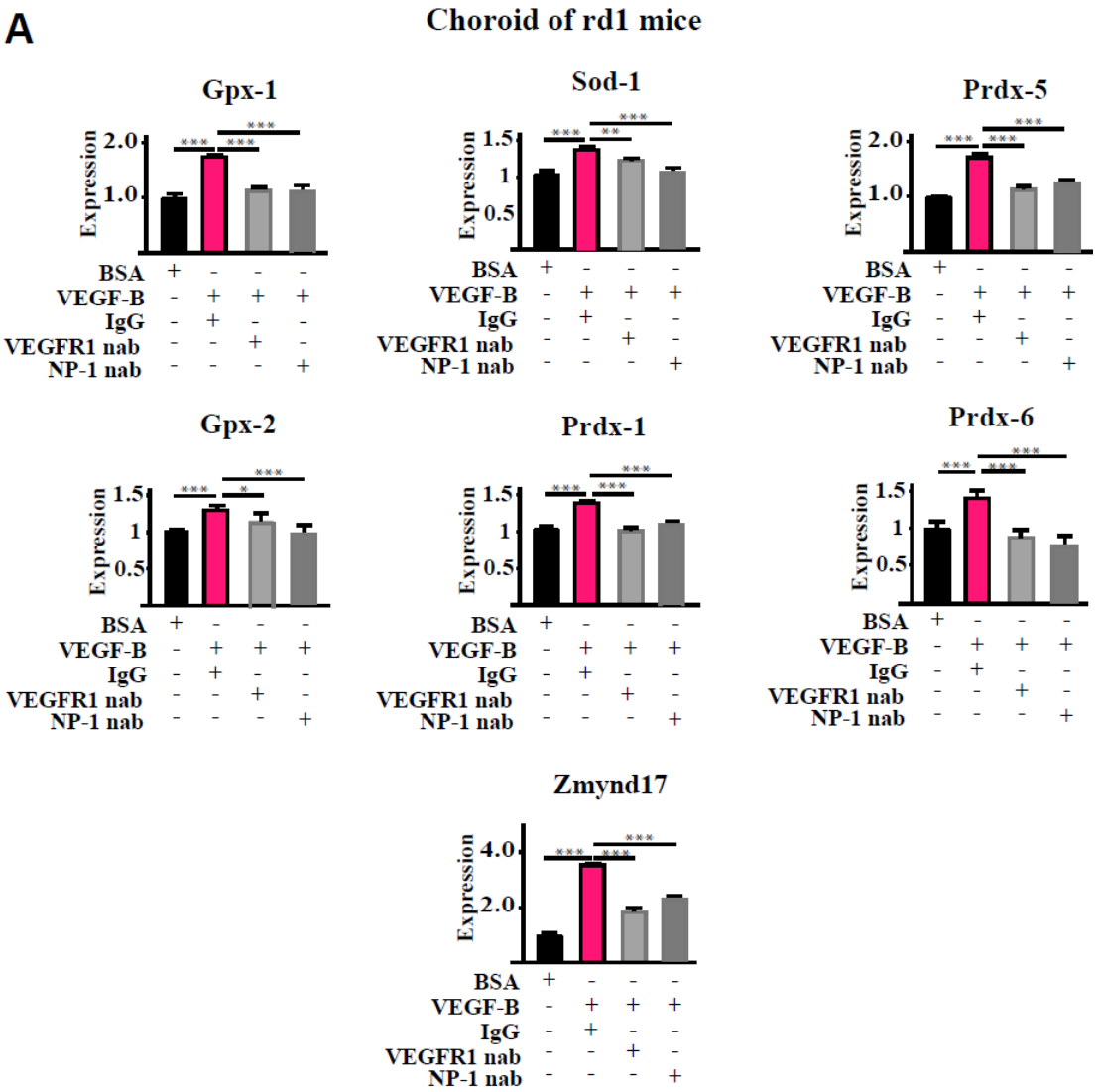

Fig. S5. The anti-oxidative effect of VEGF-B is mainly fulfilled via VEGFR1

(**A**) In the choroids of rd1 mice, real-time PCR results show that co-administration of Vegfr-1 nab completely abolished the upregulatoryeffect of VEGF-B on the expression of many anti-oxidative genes, while Np1 nab only partially diminished the effect of VEGF-B(n = 6, P< 0.001, 0.01 and 0.05).

SI Appendix

Materials and Methods

VEGF-B deficient mice and analysis of retinal thickness

All animal experiments were approved by the animal research ethics committee at the Zhongshan Ophthalmic Center, Sun Yat-Sen University, or by the Animal Care and Use Committee at the NEI/NIH. The animals were handled in accordance with the approved guidelines and regulations. *Vegf-b* deficient mice have been described previously (1, 2).

The procedures for analysis of retinal thickness have been described previously (3). Briefly, the eyes of *Vegf-b* deficient and littermate wild-type control mice were harvested at different ages. To ensure that the same locations of the eyes were analyzed and compared, a mark was placed at 12:00 o'clock position of the corneal limbus of each eye. The eyes were then embedded in Optimal Cutting Temperature (OCT, CrystalgenInc.) compound. Ten micrometer frozen sections were cut in parallel to the 12:00 o'clock meridian through the optic nerve and fixed in 4% paraformaldehyde and H&E stained. Retinal layer thickness was measured in a blinded manner regarding the genotypes at six locations of each eye: 25% (S1), 50% (S2), and 75% (S3) of the distance between the superior pole and the optic nerve, and 25% (I1), 50% (I2), and 75% (I3) of the distance between the inferior pole and the optic nerve. Only the sections with the optic nerve visible were used for analysis. When the images were captured, the whole retinal layer was included in the microscopic field and placed in parallel to the

bottom line to capture the same regions of the retinae. The thickness of the different retinal layers was measured at 200X magnification.

VEGF-B neutralizing antibody treatment

For the treatment of VEGF-B neutralizing antibody, a neutralizing antibody against mouse Vegf-b (2 ug/eye) (4)(or 20 ug/eye, R&D, AF590) or the same amount of control IgG was injected into the vitreous of 8-week old C57Bl6 mice. After 5 or 7 days, the eyes were harvested and sectioned using procedures described above. Apoptotic cells were detected using the terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) procedure according to the manufacturer's protocol (Roche Diagnostics). The numbers of $TUNEL^{+}$ nuclei per high power field were counted throughout the whole retinal section parallel to the 12:00 o'clock meridian through the optic nerve.

Treatment of rd1 mice with VEGF-B protein

The rd1/rd1 (FVB/NJ) mice as a model for retinitis pigmentosa (5, 6) were obtained from the Jackson Laboratory (Bar Harbor, ME, USA). VEGF-B_{167} protein (PeroTech, 500 ng/eye)(7) or the same amount of BSA were injected into the vitreous of postnatal day 11 (P11) mice. The injections were repeated every five days at P16, and P21. At P26, the eyes were harvested and processed as described above. The sections were stained using an H&E method. Retinal thickness was measured at six different locations as described above. Moreover, to test

a dose-response and the specificity of VEGF-B, we have also used a different dose (250 ng/eye) and used PlGF as a control, since it binds the same receptors (VEGFR1 and NP1) as VEGF-B.

Immunofluorescence staining and TUNEL assay

To visualize the rod and cone photoreceptors, the sections were incubated in rod photoreceptor-specific monoclonal mouse antibody rho-4D2 (1/100 dilution in blocking buffer; kindly provided as a gift by Dr. Robert Molday, University of British Columbia, Vancouver BC, Canada) (8) and peanut agglutinin (PNA) respectively. DAPI (Sigma) was used for nuclear staining. Sections were analyzed using Imager microscope (Carl Zeiss). TUNEL staining was performed according to the manufacturer's instructions (Roche).

PCR array analysis

The high throughput mouse RT^{2}profiler™ PCR array (SuperArray, Frederick, MD) was used to investigate the expression of eighty-four oxidative stress and antioxidant defense genes with five housekeeping genes as controls according to the manufacturer's protocol. The complete list of the genes analyzed can be found at http://www.sabiosciences.com/rt_pcr_product/HTML/PAMM-065Z.html. Briefly, total RNA was extracted from VEGF-B- or BSA-treated retinae of rd1/rd1 mice (FVB/NJ, Jackson lab) using the Trizol reagent (Invitrogen life technologies) according to the manufacturer's instructions. The extracted RNA was treated with RNeasy MinElute™ Cleanup Kit (Qiagen) to remove any potential contamination of genomic DNA according to the manufacture's protocol. One microgram of total RNA was reverse transcripted into cDNA using the RT^2 PCR array First Strand Kit (SuperArray Bioscience Corporation, Frederick, MD). The PCR array was performed using an ABI 7500 system (Applied Biosystems) and the follow program: an initial 10-minute step at 95°C followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. The data were analyzed using the web portal

(http://www.sabiosciences.com/pcr/arrayanalysis.php) and the gene expression fold changes determined using the 2-ΔΔCT method (9).

Microarray assay

The microarray analysis was performed as described previously (2). Briefly, primary mouse aortic artery smooth muscle cells (SMCs) were treated with human VEGF-B_{167} (100 ng/ml, PeproTech) for 6 hours under a hypoxic (1% oxygen) condition. Microarray assay was performed using the Whole Mouse Genome Oligo Microarray (Agilent Technologies). The whole microarray assay, including the treatment of the SMCs with VEGF-B_{167}, was repeated twice independently. The threshold of differential gene expression was set at greater than two-fold up-regulation. Analysis of the differentially expressed genes was performed using several different tools, including the WebGestalt, BRB microarray tools and the Ingenuity Pathways Analysis (IPA).

Real-time PCR and Gpx1 knockdown

For the real-time PCR assay, total RNA was isolated using the RNeasy Mini-kit (Qiagen) according to the manufacturer's instruction. 2 μg of total RNA was used for cDNA synthesis using the SuperMix kit (Invitrogen) and used for the real-time PCR reaction using an ABI Prism 7500 HT Sequence Detection System (Applied Biosystems). All experiments were performed in triplicates and repeated at least twice as described previously (2, 10).

To test whether loss of Gpx1 by shRNA knockdown could abolish the effect of VEGF-B, Gpx1 shRNA (Open Biosystems-GE Healthcare, RMM3981-97064816, 1 ug/eye) or the control vector pLKO.1 (Open Biosystems-GE Healthcare, catalog number RHS4080, 1 ug/eye) were injected into the vitreous of P7 rd1/rd1 mice together with the in vivo transfection reagent in vivo-jetPEI™ (Polyplus Transfection). After two days, human recombinant VEGF-B_{167} protein (PeproTech, catalog number 96-100-20B-100, 500 ng/eye) was injected

into the vitreous. The retinae were harvested at P11 for real-time PCR analysis. The primers used for real-time PCR are listed in SI Appendix, Tables S1-S3.

Western blot

Western blot was carried out as described previously (10). Briefly, the tissue or cell samples were homogenized in RIPA lysis buffer (50 mM HEPES pH 7.4, 150 mMNaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS). The total lysates were separated using a NuPAGE 10% Bis-Tris gel and transferred to a PVDF membrane (Invitrogen). The membranes were incubated with the following antibodies: anti-VEGF-B (MAB751, R&D), anti-Gpx1 (Novus, NBP1-33620), anti-Sod1 (Abnova, PAB1193), anti-Sod2 (AF3419, R&D), Rho-4D2 (8), anti-Zmynd17 (abcam, ab170480), anti-Prdx1 (abcam, ab15571), anti-Gpx2 (Novus, NBP1-32002), anti-alpha-tubulin (Sigma, T6074-200UL) and monoclonal anti–β-actin-Peroxidase (A3854, Sigma). The secondary antibodies included an HRP-conjugated donkey anti-mouse IgG or HRP-conjugated donkey-anti-goat IgG (R&D). Membranes were developed and visualized using an enhanced chemiluminescent western blot substrate (Pierce-Thermo Fisher Scientific Inc.).

Cell culture and MTT assay

The mouse photoreceptor-derived 661W cells (ATCC) were cultured in Dulbecco's modified Eagle's medium (ScienCell, 09221) containing 10% fetal bovine serum (ScienCell, 0025) and 1% penicillin/streptomycin. The cells were seeded at 10000 cells/well in 6-well plates and cultured overnight. For Gpx1 knockdown, about 75 % confluent 661W cells (with 10% FBS) are transfected with siCTL or siGpx1 (SR405153, OriGene) for 24 hours. After 24 hours, the cells were changed to serum-free medium and treated with VEGF-B_{167} protein (PeproTech, 100-20B, 100 ng/ml) or BSA (100 ng/ml) for two hours. Then, H_2O_2 (sigma, 323381) was added to a final concentration of 0.1% for 20 minutes, after which the medium was discarded and changed to serum-free medium with VEGF-B_{167} protein (100 ng/ml) or BSA (100 ng/ml) for 24 hours, and MTT

(3-(4, 5-dimethylthiazol-2-yl) 2, 5-diphenyltetrazolium bromide, Sigma) assay was performed to check cell viability according to the manufacturer's instruction.

Statistics

Two tailed Student's t-test was used for statistical analysis. Difference was considered statistically significant when $P < 0.05$. The data are represented as mean ± SEM of the number of the determinations. Assays using cultured cells were performed in triplicates.

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Table S1. Primers for anti-oxidative genes

Gene ID	Gene Symbol	Primer sequence
NM_008160	Gpx1- forward (mouse)	GGGACTACACCGAGATGAACG
	Gpx1- reverse (mouse)	CCGCAGGAAGGTAAAGAGC
NM_011434	SOD1- forward (rat, human and mouse)	GTGTGCGTGCTGAAGGGCG
	SOD1- reverse (rat, human and mouse)	CTTCATTTCCACCTTTGCCC
NM_012021	Prdx5- forward (rat and mouse)	GGCAGGCAGAGCAGGCCG
	Prdx5- reverse (rat, human and mouse)	GGTGAGGCCTGTGCCATC
NM_177256	Prdx6-rs1- forward (mouse)	CGGTTGCCACCCCAGTT
	Prdx6-rs1- reverse (mouse)	TGCCACGATCTTTCTACGGAC
NM_153162	Txnrd3- forward (rat, human and mouse)	GGAACTTGATCAAGTTGATG
	Txnrd3- reverse (rat and mouse)	GGCTGCCTGATGCATCAGC
NM_013671	Sod2- forward (rat, human and mouse)	CAGATCATGCAGCTGCACC
	Sod2- reverse (rat, human and mouse)	CATTCTCCCAGTTGATTACATTC
NM_010343	Gpx5- forward (mouse)	GGGATGTAAATGGTGAAAACGA
	Gpx5- reverse (mouse)	CCAGCGCATGACAGGGAT
NM_029104	Zmynd17- forward (rat, human and mouse)	GGCACTGTAAGAGGTGCAG
	Zmynd17- reverse (rat and mouse)	CGTGGGAAGCACCAACCAC
NM_030677	Gpx2- forward (rat, human and mouse)	GTGCTGATTGAGAATGTGGC
	Gpx2- reverse (rat, human and mouse)	CTATGAGGAACTTCTCAAAGTTC
NM_015762	Txnrd1- forward (human and mouse)	GATGGGGTCTCGGAGGAAC
	Txnrd1- reverse (rat, human and mouse)	GTAAGGCAAGGAGAAAAGATC
NM_011034	Prdx1- forward (rat, human and mouse)	CACGGAGATCATTGCTTTCAG
	Prdx1- reverse (rat, human and mouse)	GGTATCACTGCCAGGTTTCC
NM_145451	Gpx6- forward (rat, human and mouse)	GGTGGATTGCAACAAAGGGG
	Gpx6- reverse (rat and mouse)	CTTCATGGGATCCCAGAAGAG

Table S2. Primers for oxidative stress genes

Gene ID	Gene Symbol	Primer sequence
NM_008969	Ptgs1- forward (rat and mouse)	GAATGCCACCTTCATCCGAG
	Ptgs1- reverse (rat and mouse)	CCAGCACCTGGTACTTAAG
NM_015760	Nox4- forward (human and mouse)	CCTCTTCTTTGTCTTCTACATG
	Nox4- reverse (human and mouse)	GCACAAAGGTCCAGAAATCC
NM_010877	Ncf2- forward (rat and mouse)	CAAAAGCATCAACAGAGACAAG
	Ncf2- reverse (rat and mouse)	GCTGACTGTGGCTGCAGAG
NM_009417	Tpo- forward (rat and mouse)	GGAGGGAGACCTTCCCAC
	Tpo- reverse (rat and mouse)	GTGTCCACCTGCAAATTAC
NM_133819	Ppp1r15b- forward (mouse)	CCGAGTGCAGATGAAGCAG
	Ppp1r15b- reverse (rat, human and mouse)	GAATTCGTTTCTGGAACCTGC

Table S3. Primers for Flt1

Gene ID	Gene Symbol	Primer sequence
NM_010228	Flt1- forward (mouse)	GCTCTGATGACCGAACTCAA
	Flt1- reverse (mouse)	ATTCCACGATCACCATCAGA