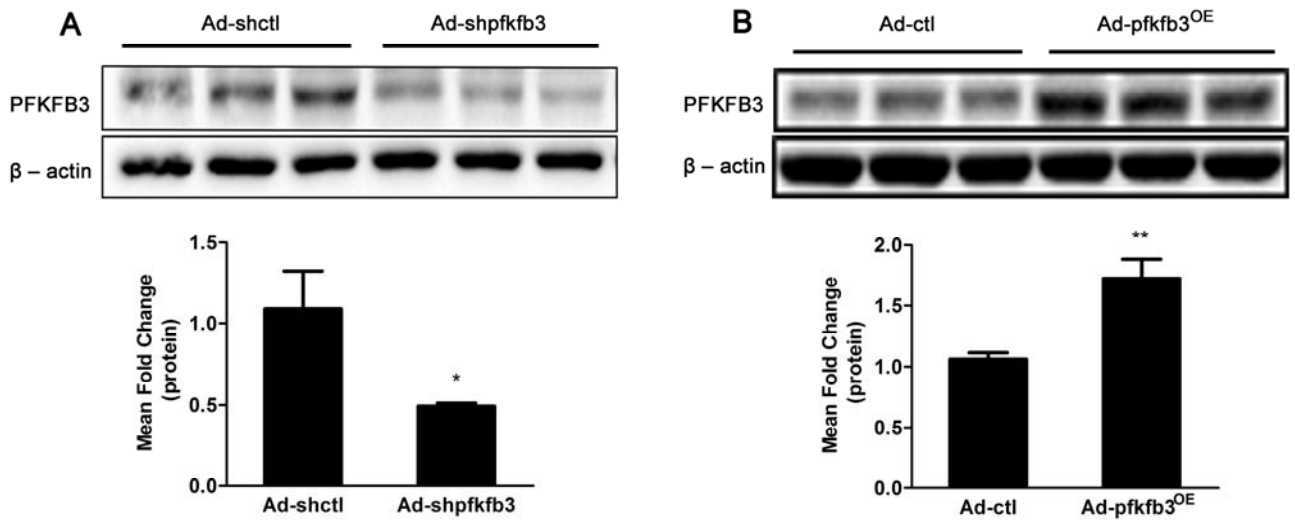
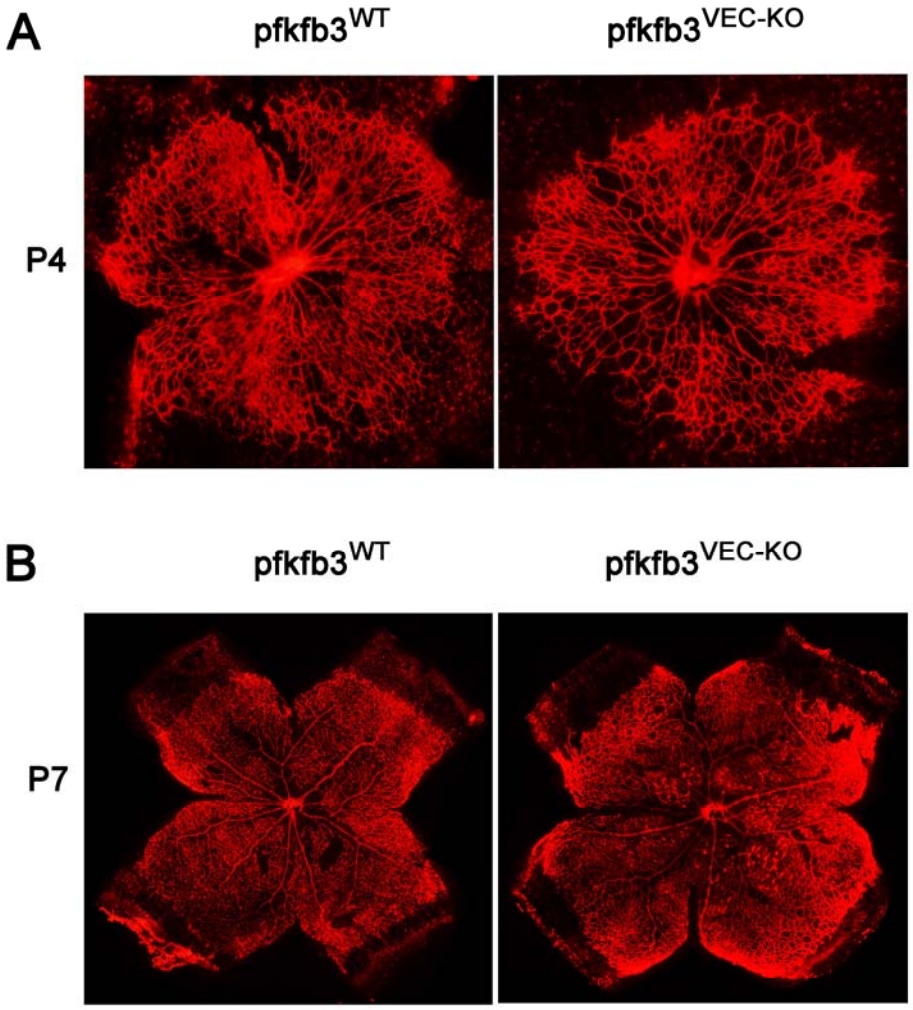
SUPPLEMENTAL DATA



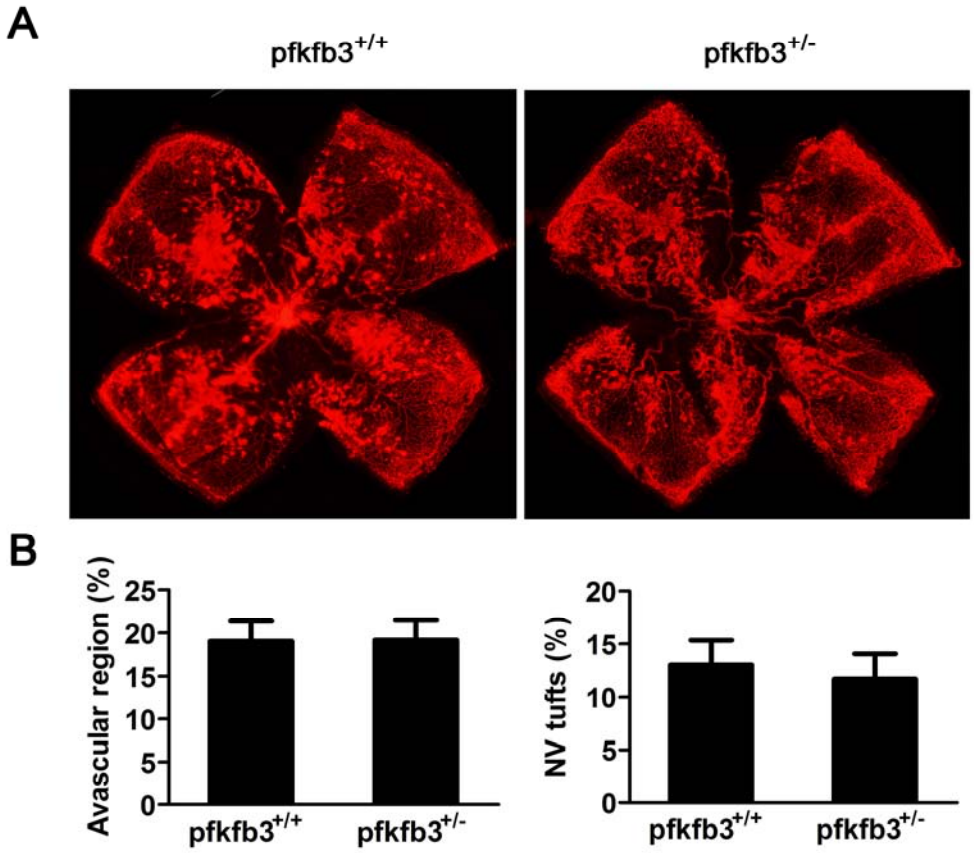
Supplemental figure I. PFKFB3 knockdown or overexpression in HUVECs.

HUVECs were transfected with Ad-shctl, Ad-shpfkfbs (**A**), Ad-ctl, and/or Ad-pfkfb3 (**B**) for 36 h and then collected for analysis of PFKFB3 expression using Western blotting. For bar graphs, data are the mean ± SD, n = 3. *, $P < 0.05$ and **, $P < 0.01$ Ad-shpfkfb3 vs. vs. Ad-shctl or Ad-pfkfb3^{OE} vs Ad-ctl.



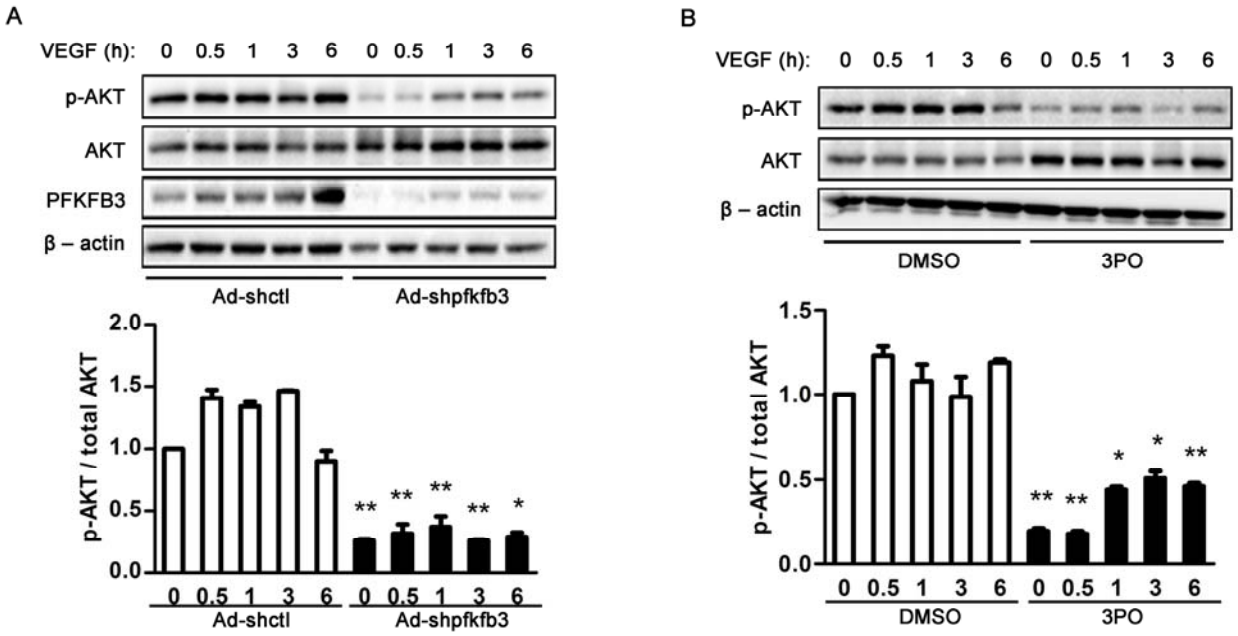
Supplemental figure II. The effects of PFKFB3 deficiency on normal postnatal retinal development.

A, Isolectin staining of whole-mount retinal samples of PFKFB3$^{flox/flox}$ and PFKFB3$^{flox/flox}$cdh5^{cre} mice at P4 (n = 8 mice per genotype). B, Isolectin staining of whole-mount retinal samples of PFKFB3$^{flox/flox}$ and PFKFB3$^{flox/flox}$cdh5^{cre} mice at P7 (n = 8 mice per genotype).



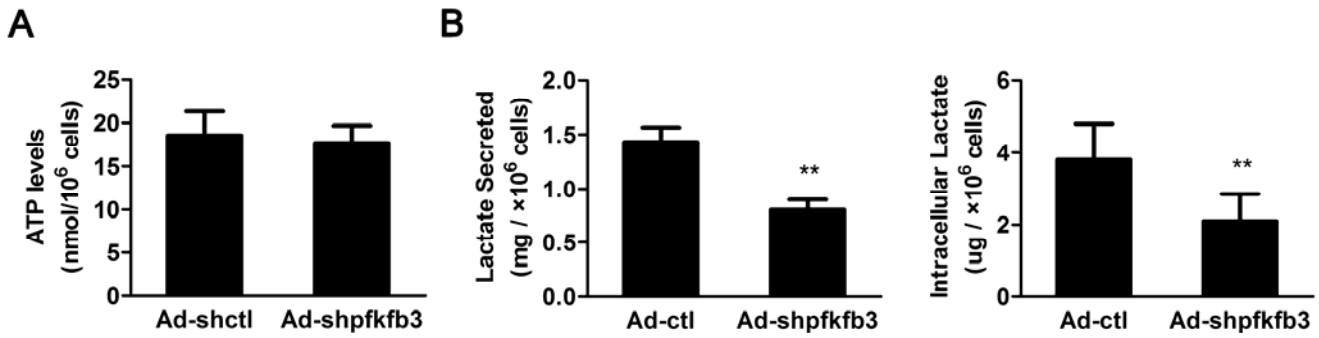
Supplemental figure III. The oxygen-induced retinopathy in pfkfb3$^{+/+}$ and pfkfb3$^{+/-}$ mice.

A, Representative images of isolectin-stained whole-mount retinal samples of PFKFB3$^{+/+}$ and PFKFB3$^{+/-}$ OIR mice at P17. B, Quantification of the neovascular tuft (NVT) and avascular areas (n = 8 mice per genotype).



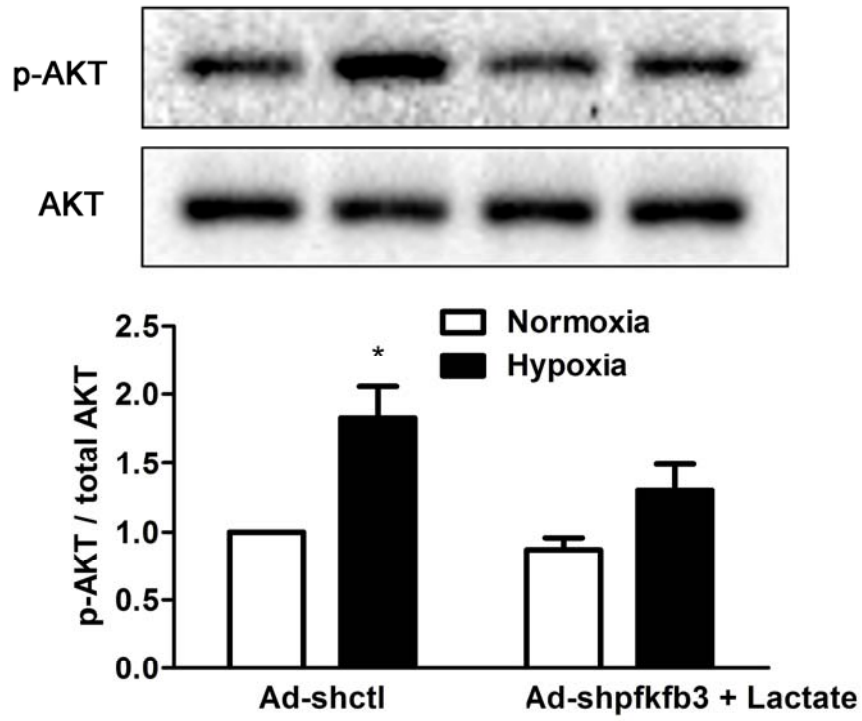
Supplemental figure IV. AKT as downstream event of PFKFB3 in endothelial cells following VEGF treatment.

A, Western blot analysis of p-AKT and AKT in control and PFKFB3-knockdown HUVECs upon VEGF treatment for 0, 1/2, 1, 3 or 6 hrs. B, Western blot analysis of p-AKT and AKT in control and 3PO-treated HUVECs exposed to VEGF for 0, 1/2, 1, 3 or 6 hr. All images shown are representative, and data are the mean ± SD (n = 3 independent experimental groups). *, $P < 0.05$ and **, $P < 0.01$ Ad-shpfkfb3 vs. Ad-shctl or 3PO vs DMSO under the same condition.



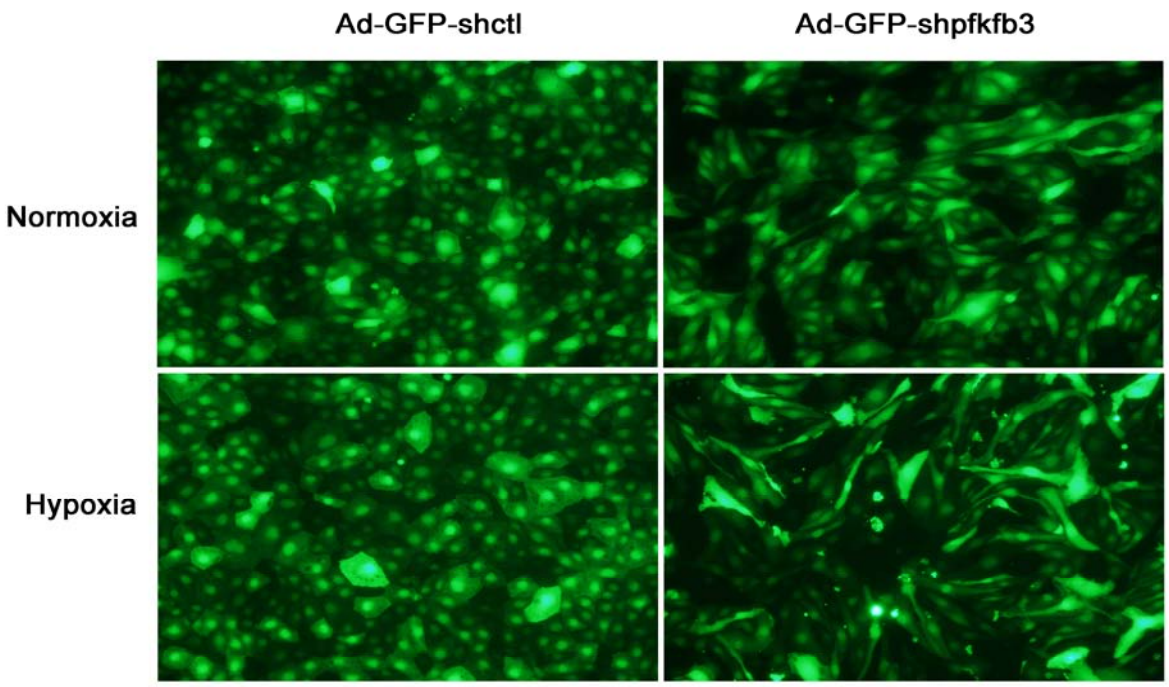
Supplemental figure V. Lactate production in PFKFB3-knockdown HUVECs under hypoxic conditions.

A, Quantification of ATP levels in PFKFB3-knockdown HUVECs and control cells after 24 h incubation. B, Quantification of secreted lactate levels and intracellular lactate levels in PFKFB3-knockdown HUVECs and control cells after 72 h hypoxic treatment. Data are the mean ± SD (n = 3). **, $P < 0.01$ Ad-shpfkfb3 vs. Ad-shctl under the same condition.



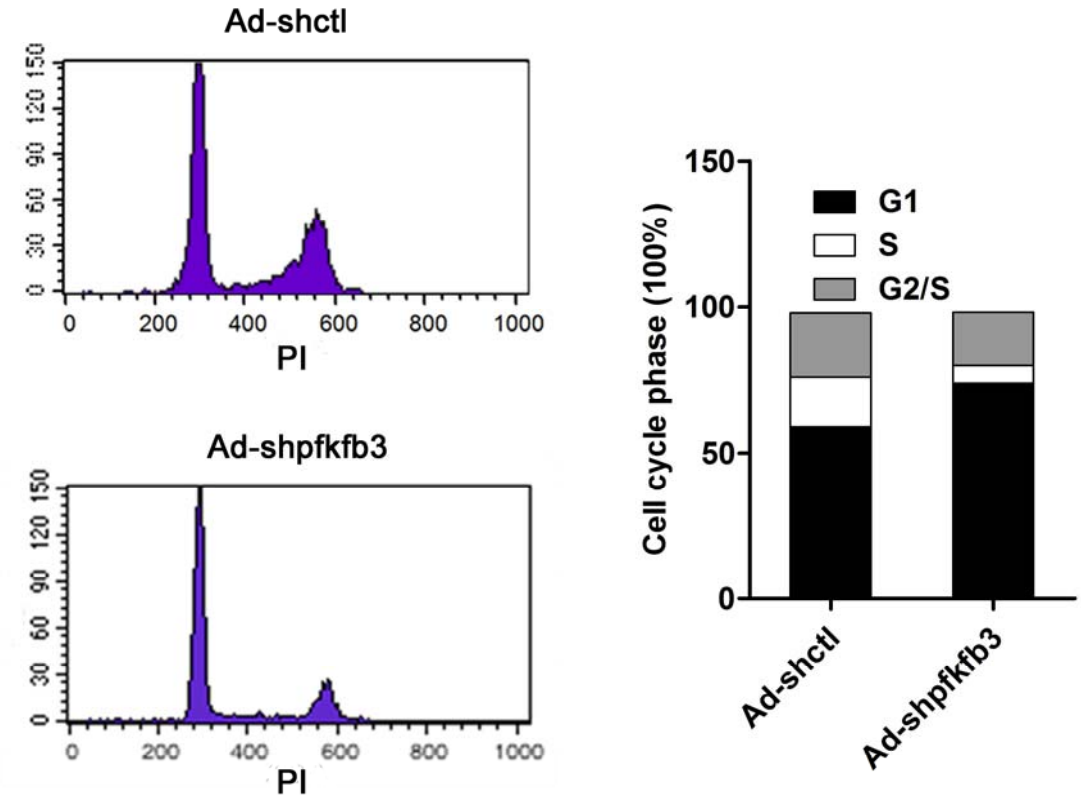
Supplemental figure VI. Western blot analysis of p-AKT and AKT in PFKFB3-knockdown HUVECs upon hypoxia.

PFKFB3-knockdown HUVECs and control cells were exposed to normoxia and/or hypoxia for 6 h. For the PFKFB3-knockdown HUVECs, 10 mM sodium lactate was added 1 h before exposed to normoxia and/or hypoxia. Data are the mean ± SD (n = 3). *, $P < 0.05$ hypoxia vs. normoxia in control HUVECs.



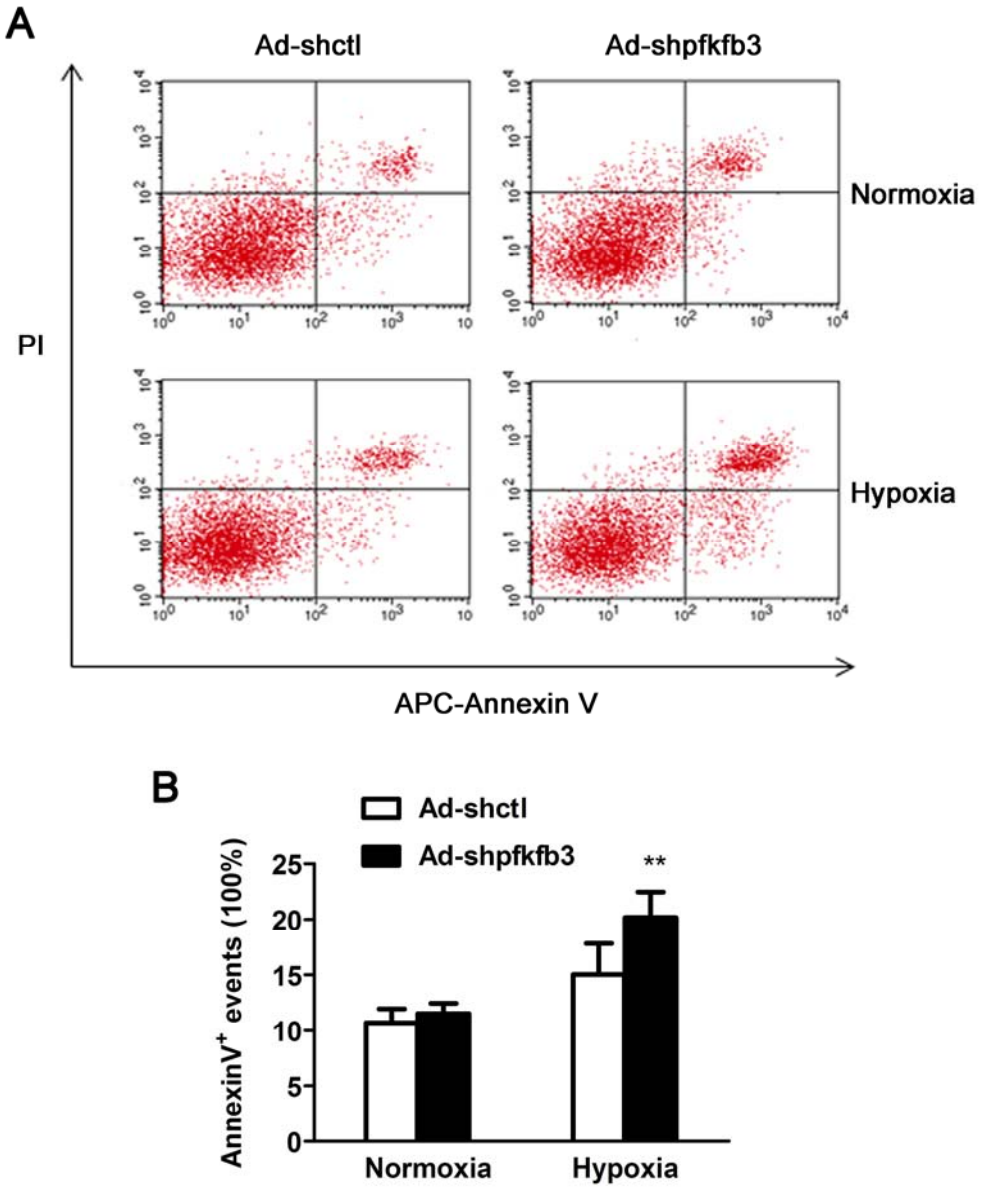
Supplemental figure VII. Morphology of PFKFB3-knockdown HUVECs.

Representative photomicrographs of cultured PFKFB3-knockdown HUVECs and control cells exposed to normoxia or hypoxia (0.5% oxygen) for 24 h.



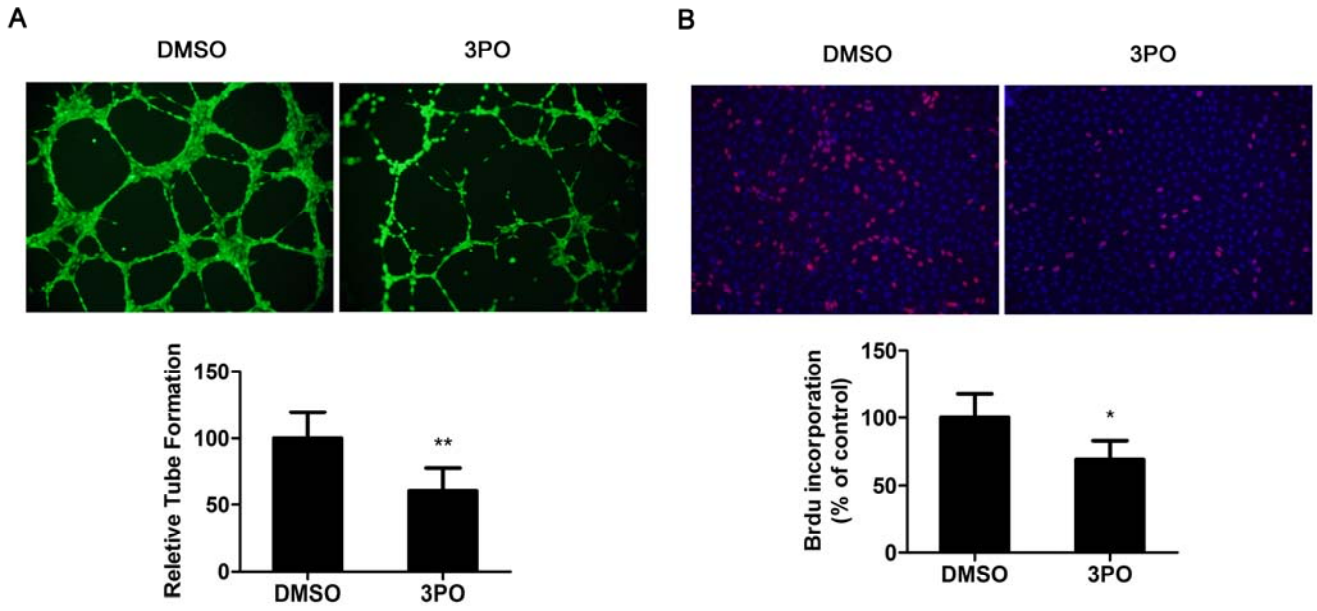
Supplemental figure VIII. **The cell cycle of PFKFB3-knockdown HUVECs under hypoxic conditions.**

PFKFB3-knockdown HUVECs and control cells were synchronized in G0/G1 by total FBS depletion for 16 h and then exposed to complete medium for an additional 24 h under hypoxic condition (0.5% oxygen).

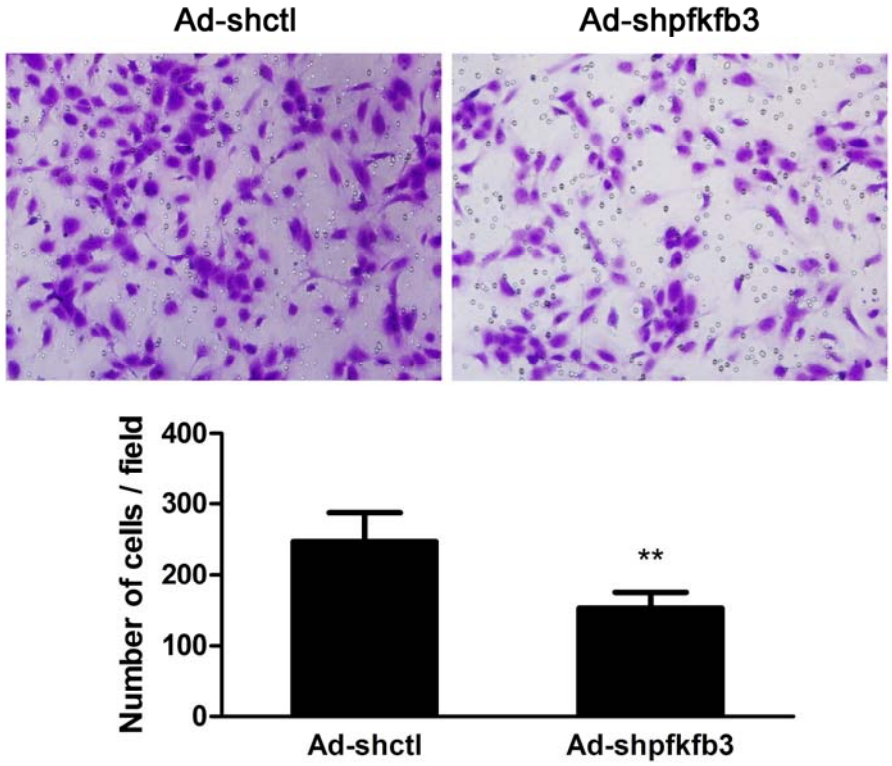


Supplemental figure IX. Involvement of PFKFB3 in endothelial apoptosis.

A, Flow cytometric analysis of apoptosis in PFKFB3-knockdown HUVECs and control cells under a normoxic or hypoxic environment. B, Quantification of the annexin V^+ HUVEC ratio for each group. **, $P < 0.01$ Ad-shpfkfb3 vs. Ad-shctl under the same condition.



Supplemental figure X. Involvement of PFKFB3 in endothelial proliferation and tube formation. A, Representative images and quantification of tube formation in 3PO-treated HUVECs and control cells cultured in growth factor-deprived Matrigel. B, BrdU staining of proliferating HUVECs. 3PO-treated cells and control cells were synchronized in G0/G1 by total FBS depletion for 16 h and then incubated with complete medium for an additional 24 h. For bar graphs, data are the mean ± SD, n = 3. *, $P < 0.05$ and **, $P < 0.01$ 3PO vs. control. All images shown are representative.



Supplemental figure XI. Involvement of PFKFB3 in endothelial migration.

Representative images and quantification of endothelial migration. PFKFB3-knockdown HUVECs and control cells cultured in a transwell migrated to cell medium with hVEGF at 20ng/mL. For bar graph, data are the mean ± SD, n = 3. **, $P < 0.01$ for Ad-shpfkfb3 vs. Ad-shctl.

Supplemental table I. Primer sequences for QRT-PCR analysis

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
Mouse pfkfb3	GATCTGGGTGCCCGTCGATCACCG	CAGTTGAGGTAGCGAGTCAGCTTC
Human pfkfb1	CTCCATCTACCT TTGCCGACA	GCCCTGGGACTGAATGAAGTT
Human pfkfb2	CACCAATACAACCCGGGAGA	GCAGCAATGACATCAGGATCA
Human pfkfb3	CTCGCATCAACAGCTTTGAGG	TCAGTGTTTCCTGGAGGAGTC
Human pfkfb4	CCAACTGCCCAACTCTCATTG	GCGATACTGGCCAACATTGAA
Mouse HPRT	AGTGTTGGATACAGGCCAGAC	CGTGATTCAAATCCCTGAAGT
18S ribosomal RNA	GCCTCACTAAACCATCCAA	TCAGTGTTTCCTGGAGGAGTC