Supplementary Information for

An allosteric peptide inhibitor of HIF-1α regulates hypoxia-induced retinal neovascularization

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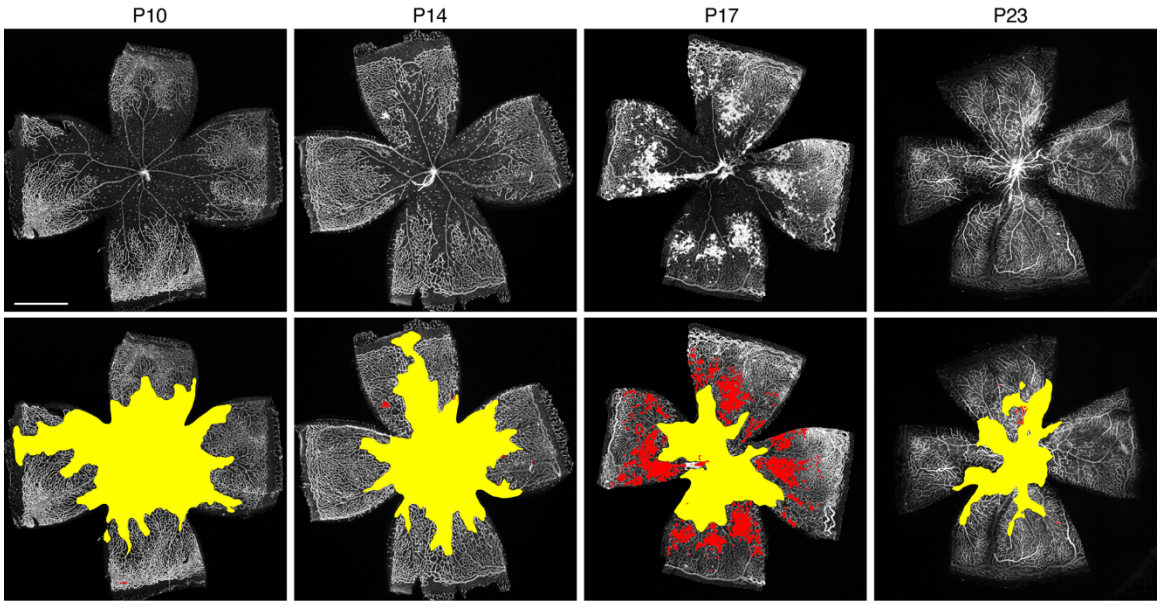
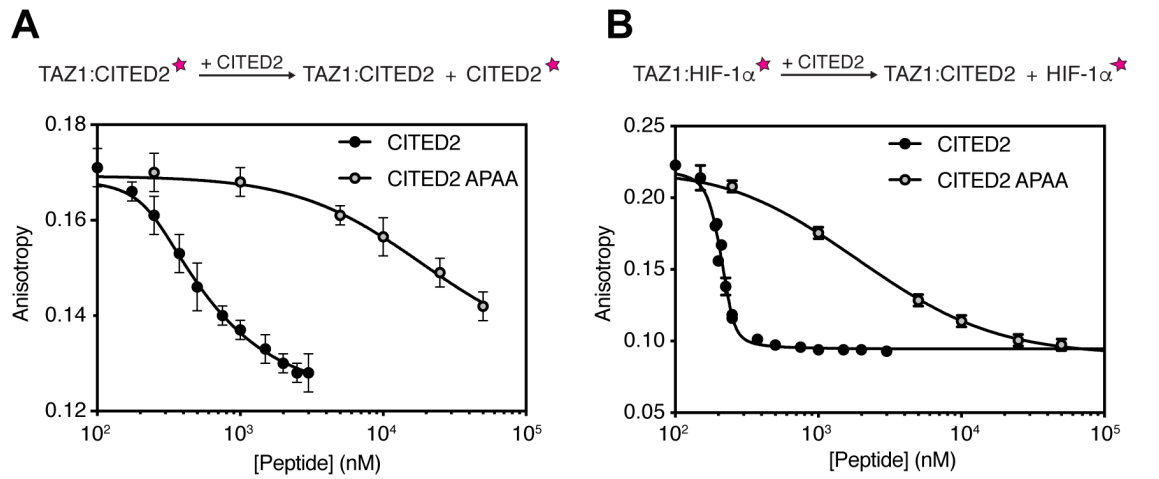


Fig. S1. Immunofluorescent staining of OIR retinas. Retinal whole mounts were prepared at P10, P14, P17, and P23 and stained with GS-lectin. The raw images for each time point are shown in the top row, with the corresponding quantified and annotated images shown below, with NV highlighted in red and VO highlighted in yellow. Scale bars: 1 mm.



Competing peptide	K_d^{app} for TAZ1
CITED2	10 ± 0.1 nM
CITED2 APAA	2.5 ± 0.2 μM

Competing peptide	Competition K_d^{app}
CITED2	0.2 ± 0.1 nM
CITED2 APAA	50 ± 10 nM

Fig. S2. The CITED2 APAA peptide is deficient in binding and competing with HIF-1α for the TAZ1 domain of CBP/p300. (A) Fluorescence anisotropy data for titration of unlabeled CITED2 and CITED2 APAA peptides into a pre-formed complex of Alexa594-labeled CITED2 and unlabeled TAZ1. A schematic of the experimental design is shown above the graph. The apparent binding affinities (K_d^{app}) of the CITED2 peptides for TAZ1 are reported below the graph. (B) Fluorescence anisotropy data for titration of unlabeled CITED2 and CITED2 APAA peptides into a pre-formed complex of Alexa594-labeled HIF-1α and unlabeled TAZ1. A schematic of the experimental design is shown above the graph. The apparent affinities of the CITED2 peptides competing with HIF-1α for TAZ1 binding (competition K_d^{app}) are reported below the graph. For (A) and (B), data for the CITED2 peptide are shown in black and for the CITED2 APAA peptide in grey. The data shown represent the mean (circles) and standard deviation (error bars) of three independent replicates.

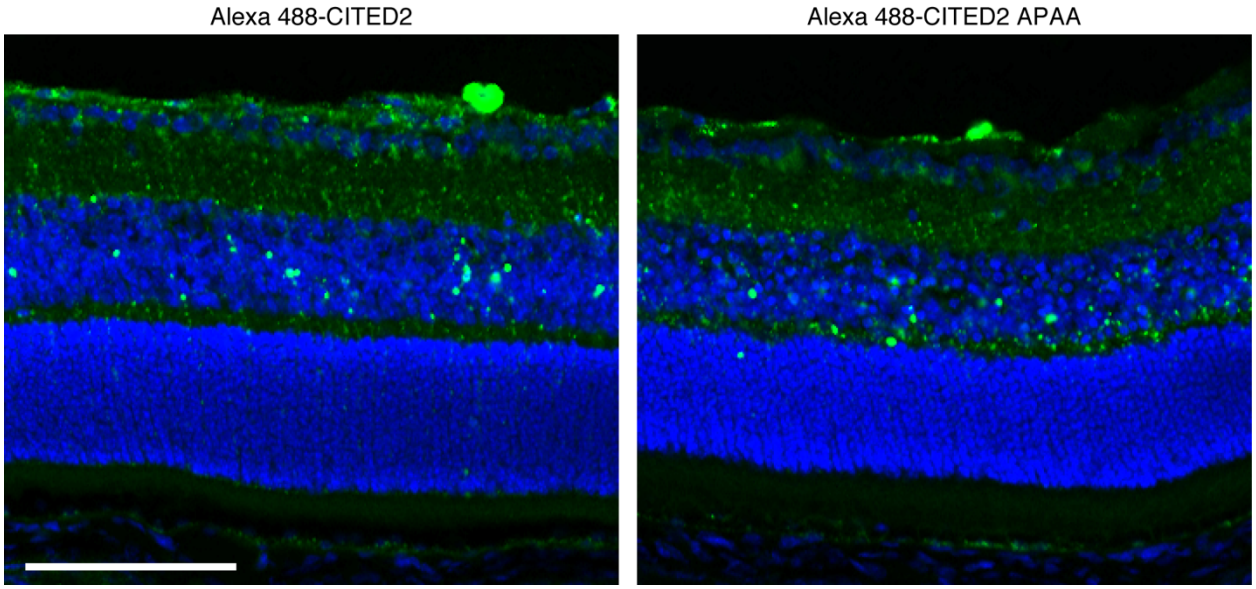


Fig. S3. The distribution of Alexa488-CITED2 and Alexa488-CITED2 APAA in OIR retinas. Retinal cross-sections were stained with DAPI 12 hours after intravitreal injection of Alexa488-CITED2 or Alexa488-CITED2 APAA. Scale bars: 100 μm.

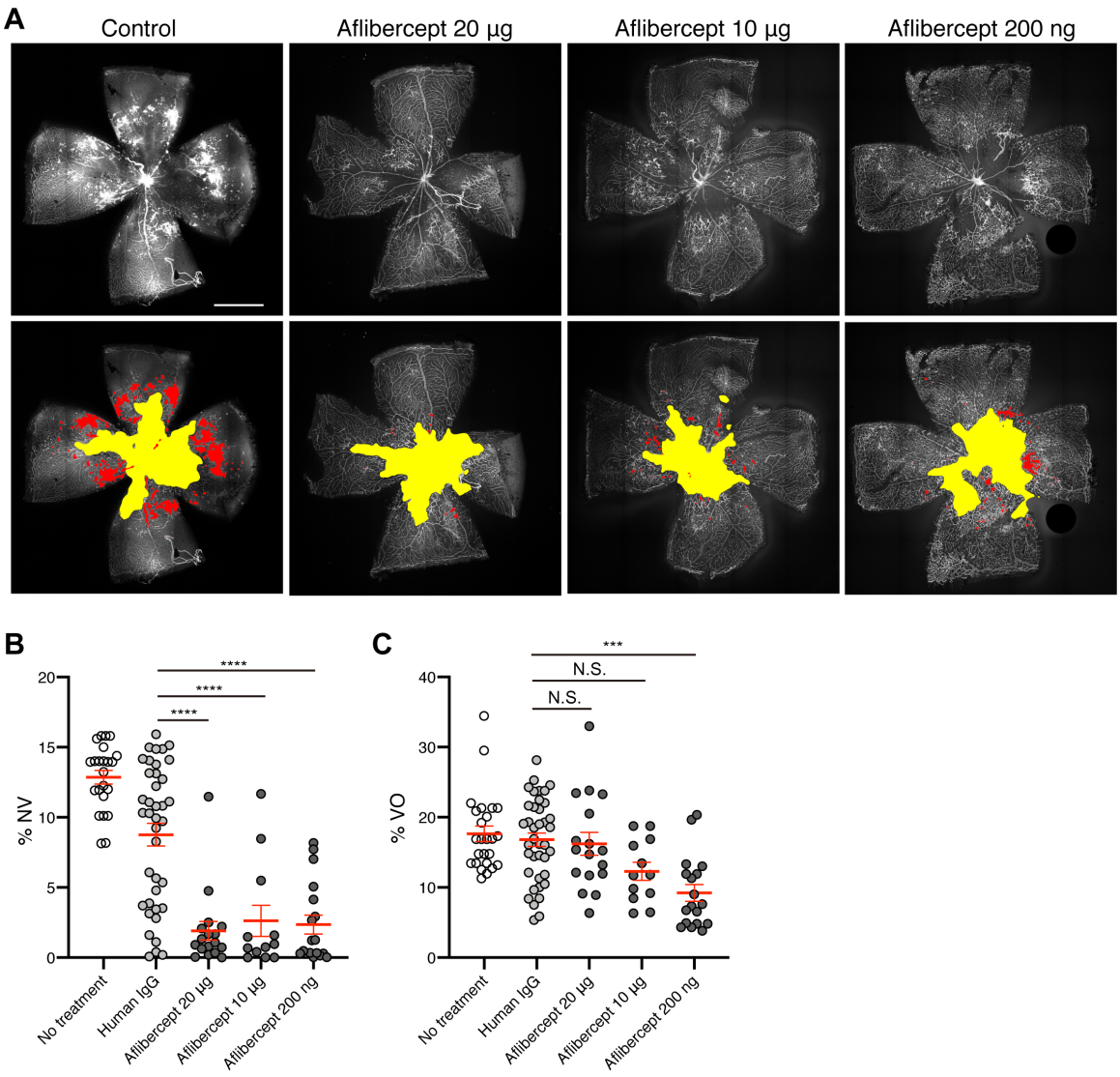


Fig. S4. Aflibercept rescues OIR-induced neovascularization but not vaso-obliteration. (A) Immunofluorescent staining of P17 OIR retinas. OIR mice were intravitreally injected with 0.5 µL of Aflibercept (20 µg, 10 µg, 200 ng) or human IgG (1 µg) as an isotype control. Retinal whole mounts were stained with GS-lectin. Representative images are shown in the top row; the same images are shown directly below with NV highlighted in red and VO highlighted in yellow as used for quantification. Scale bars = 1 mm. (B) Quantification of the percentage of NV area in whole retinas. (C) Quantification of the percentage of VO area in whole retinas. For (B) and (C), n > 8 per group. *P*-values were calculated using one-way ANOVA with Tukey's multiple comparisons test. ****$P < 0.0001$. N.S., not significant. The mean and SEM are shown in red.

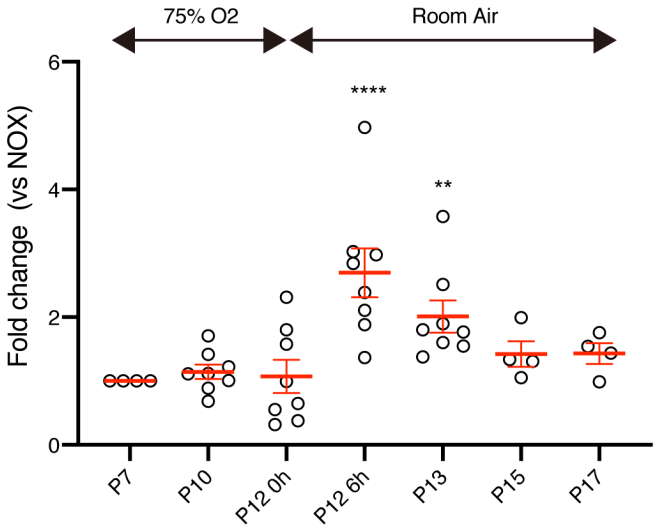


Fig. S5. The expression level of *Cited2* mRNA at various time points of the OIR experiment. Total RNA was isolated from OIR retinas. The expression levels are normalized to *Cited2* expression in retinal cells from age-matched normoxic controls (n > 4 for each time point). The mean and SEM are shown in red. *P*-values were calculated using a two-way ANOVA test. ** $P < 0.01$, **** $P < 0.0001$.

Table S1. Primer sequences for PCR.

36b4 forward	CTGTGCCAGCTCAGAACACTG
36b4 reverse	TGATCAGCCCGAAGGAGAAG
Vegfa forward	AACGATGAAGCCCTGGAGT
Vegfa reverse	AGGTTTGATCCGCATGATCT
Epo forward	CGACAAAGCCATCAGTGGTCTACG
Epo reverse	GCAGAAAGTATCCACTGTGAGTG
Ndufa4l2 forward	TGGCTTCATCTGCTTGGGCATG
Ndufa4l2 reverse	GTCATTGGGACTCAGGCGGTTC
Ldha forward	GGCACTGACGCAGACAAG
Ldha reverse	TGATCACCTCGTAGGCACTG
ActB forward	AGATCTGGCACCACACCTTC
ActB reverse	GGGGTGTTGAAGGTCTCAAA
Tie2 forward	CGGCCAGGTACATAGGAGGAA
Tie2 reverse	CCCCCACTTCTGAGCTTCAC
CD11b forward	CCCAGAACCTCTCAAGTGCC
CD11b reverse	CTGCAACAGAGCAGTTCAGC
Vim forward	GGCTCGTCACCTTCGTGAAT
Vim reverse	AGAAAAGGTTGGCAGAGGCA
Pax6 forward	GTTCTTCGCAACCTGGCTA
Pax6 reverse	TGAGCTTCATCCGAGTCTTCT
Glul forward	TGCCTGCCCAGTGGGAATT
Glul reverse	TATTGGAAGGGTTCGTCGCC
Gfap forward	GCACTCAATACGAGGCAGTG
Gfap reverse	GGCGATAGTCGTTAGCTTCG
Fgf11 forward	TCCTCATCCTGCTGTCCAAGGT
Fgf11 reverse	ATTCGCCTGGAGGTAGAAACCC

Table S2. Taqman assays for PCR.

ActB	Mm02619580_g1
Vegfa	Mm00437306_m1
Epo	Mm01202755_m1
Tnfa	Mm00443258_m1
Ccl2	Mm00441242_m1
Ccl3	Mm00441259_g1
Il1b	Mm00434228_m1
Cited2	Mm01188099_g1