SUPPLEMENTAL DATA

AIP1 mediates VEGFR-3-dependent angiogenic and lymphangiogenic responses

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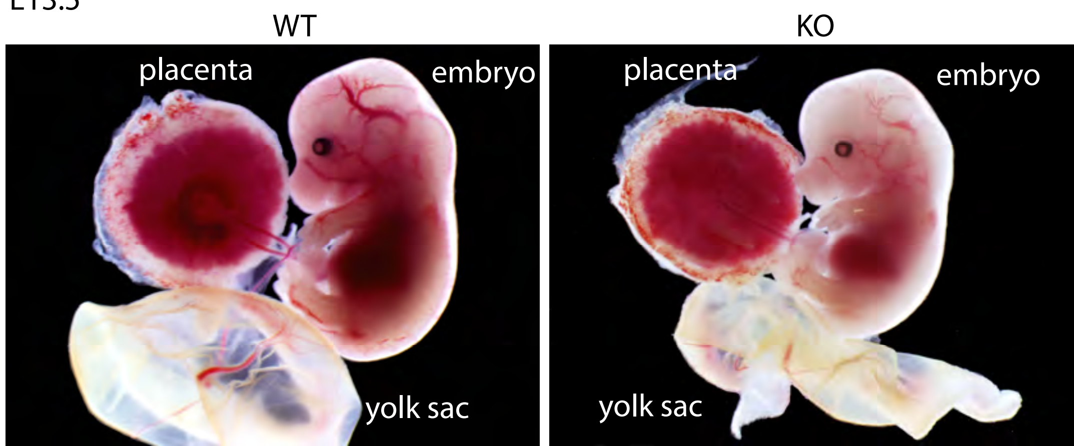

Fig.I. Effects of AIP1 deletion on embryo vascular development. WT and AIP1-KO embryos were harvested at E9.5-E16.5. Freshly dissected embryos without staining were photographed. Shown images are embryo, yolk sac and placenta at E13.5.

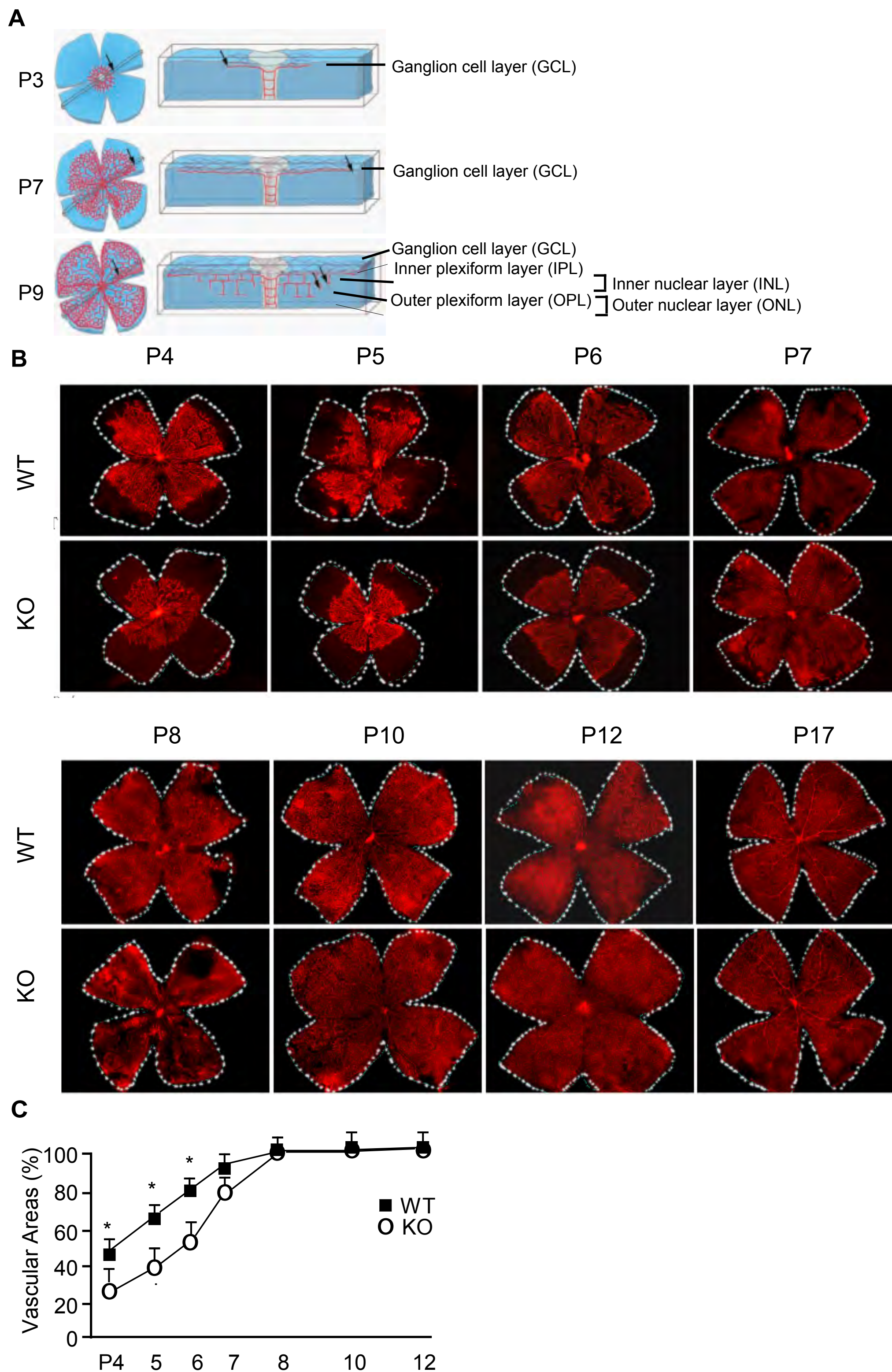

Fig.II. Effects of AIP1 deletion on retinal vascular development. A. A cartoon for retinal vascular development. The vasculatures reach to one third, two thirds and the edge of the retina on P3, P7 and P9 respectively. Intraretinal vessel growth from GCL to IPL and OPL are shown. Vessels are mainly located in the GCL at P7, and sequentially grow deeper into the inner plexiform layer (IPL) and outer plexiform layer (OPL) at P9. **B**. P4-P17 retinas were subjected to whole-mount staining with isolectin for EC. **C**. Vascularized areas were quantified as a percentage of the total retinal surface. n=10 retinas from 5 mice for each strain. Data are mean ± SEM. *, $p<0.05$ comparing AIP1-KO to age-match WT.

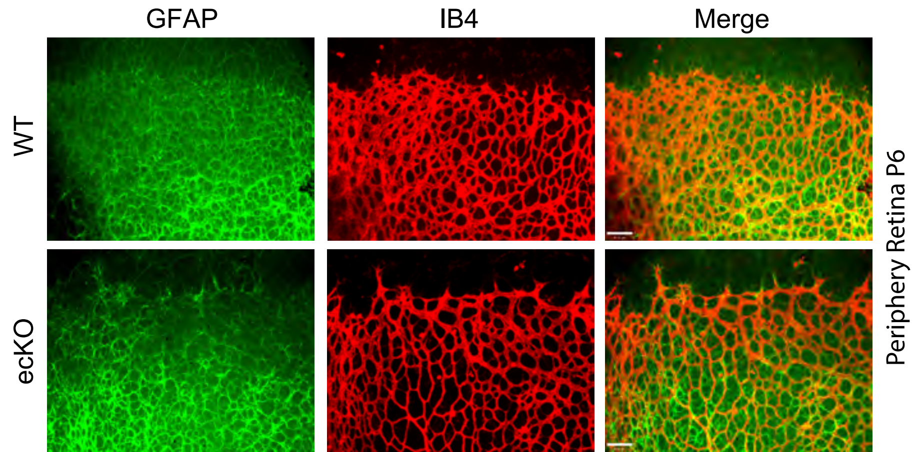

Figure III. Effects of AIP1 deletion on retinal astrocyte-EC interactions.
P6 retinas from WT (AIP1lox/lox) and the AIP1-ecKO (AIP1lox/lox:Tie2-Cre) were collected. Retinas were subjected to whole mount staining with isolectin for EC (red) and GFAP for astrocytes (green). Representative images from 3 pairs of WT and AIP1-ecKO are shown. Scale bar: 50 μm.

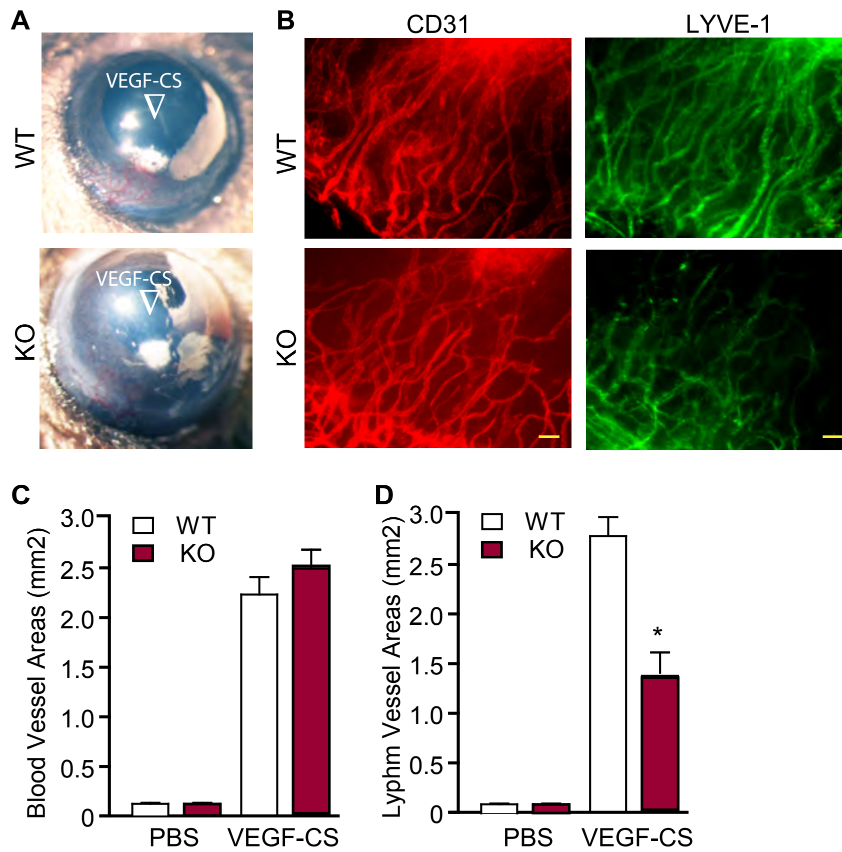

Figure IV. VEGF-CS induced cornea lymphangiogenesis is attenuated in AIP1-KO mice. Hydron pellet containing PBS or VEGF-CS was implanted into the cornea of WT and AIP1-KO mice. A. Neovascularization was assessed using stereomicroscopy on day 14 following implantation. B. Corneas were harvested on day 14. Blood and lymphatic vessels were visualized by whole-mount immunostaining with anti-CD31 and anti-LYVE-1. Scale bar, 100 μm. C-D. Blood and lymphatic vessel areas were quantified. Data are mean ± SEM, n=6 for each strain, *, $p<0.05$.

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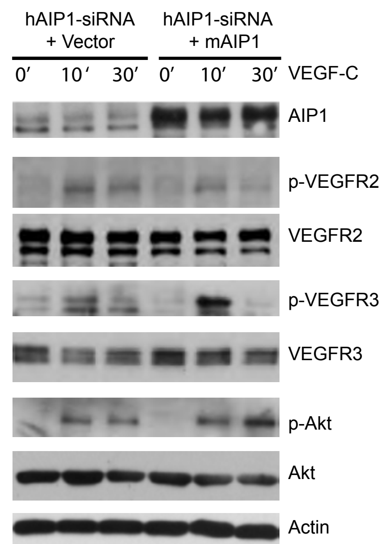

Fig.V. Overexpression of AIP1 rescues VEGF-C signaling in AIP1 knockdown cells. HLEC were transfected with human AIP1 siRNA. 24 h later, cells were transfected with murine AIP1 expression plasmid or a vector control. 24 h later, cells were treated with VEGF-C (100 ng/ml) for indicated times. Phosphorylations of VEGFR-2, VEGFR-3 and Akt were determined by Western blot with phospho-specific antibodies. Total protein levels of VEGFR-2, VEGFR-3, Akt and AIP1 were determined by Western blot with respective antibodies. β-actin was used as a loading control. Similar results were obtained from additional two experiments.

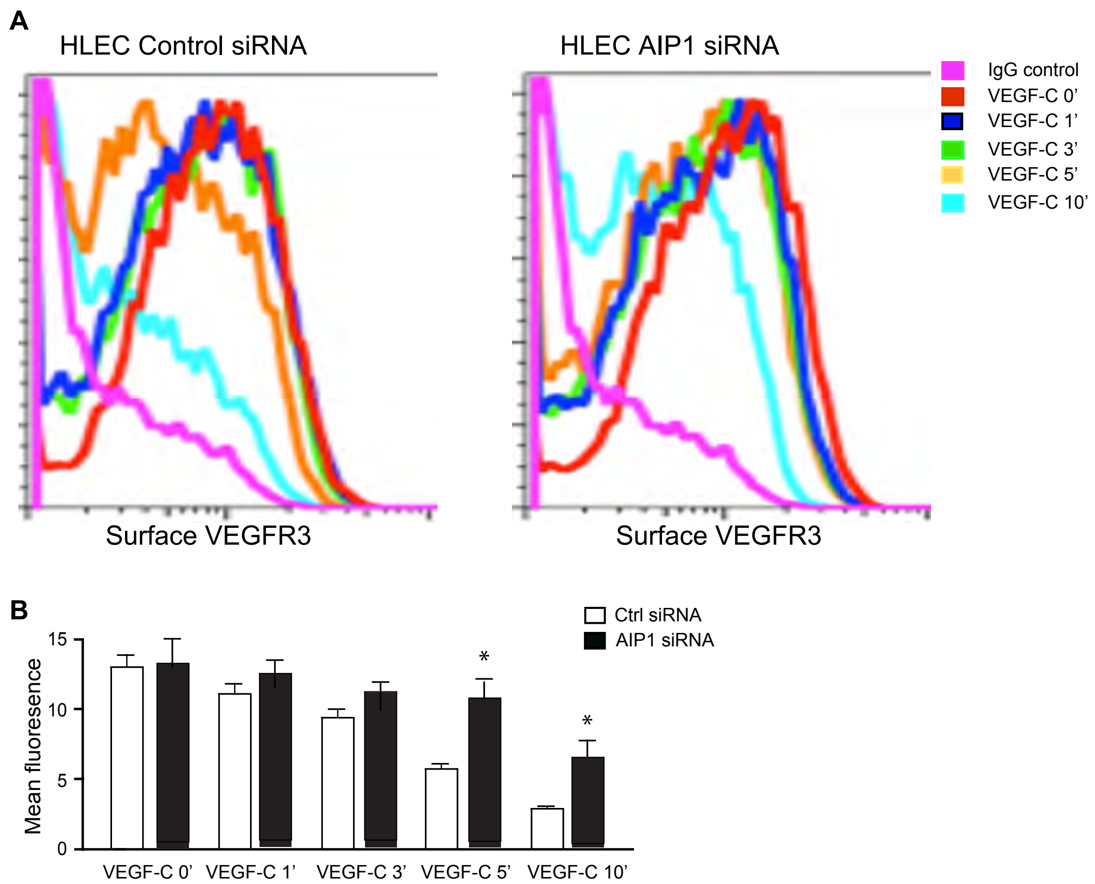

Figure VI. Effects of AIP1 knockdown on VEGFR-3 surface expression. HLEC were transfected with Ctrl or AIP1 siRNA (20 nM) for 48 h. HLEC were treated with VEGF-C (100 ng/ml) for indicated times. Surface VEGFR-3 was detected by FACS with anti-VEGFR-3. Representative FACS graphics are shown in A. Surface levels of VEGFR-3 (Mean Fluorescence intensity, MFI) are quantified in B. Data are mean ± SEM from three independent experiments. *, $p<0.05$.

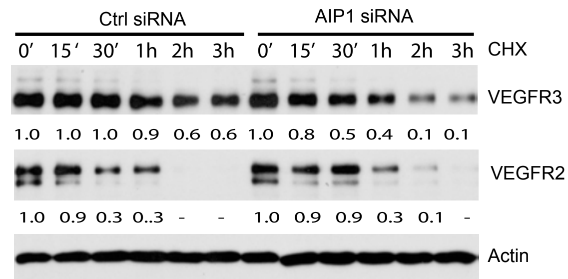

Fig.VII. Effects of AIP1 knockdown on VEGFR-3 half-life.
HLEC were transfected with Ctrl or AIP1 siRNA (20 nM), 48 h aftertransfection, HLEC were treated with cycloheximide (10 µg/ml) for indicated times. Total levels of VEGFR-3, VEGFR-2, AIP1 and β-actin were determined by Western blot with respective antibodies. Relative levels of total VEGFR-3 and VEGFR-2 are indicated below the blot with untreated Ctrl group as 1.0. Similar results were obtained from additional two experiments.