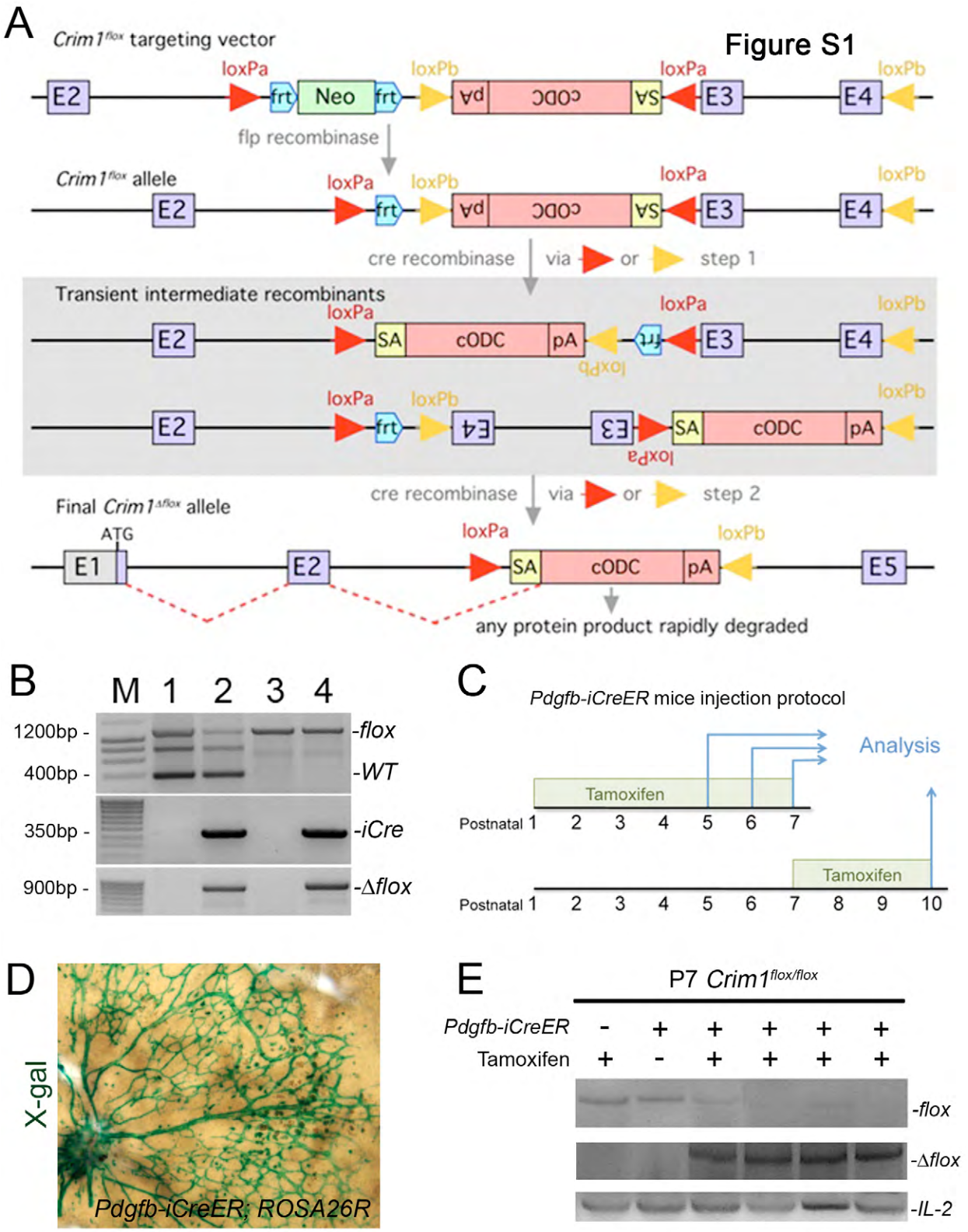
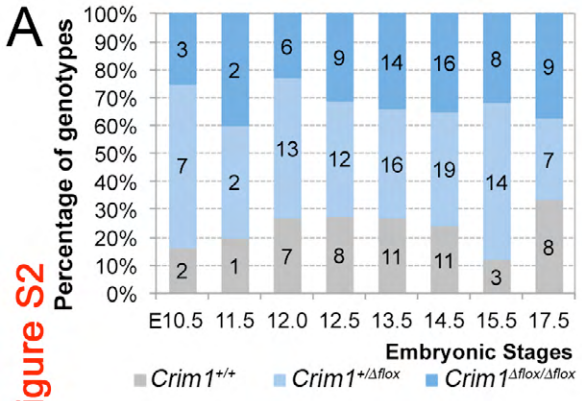



**Fig. S1. *Crim*$^{flox}$ allele design and inducing VEC specific cre activity in *PdgfbiCre* mouse line.** (A) Diagram of *Crim1*$^{flox}$ allele. (B) Genomic DNA PCR could distinguish mice with or without *Crim1*$^{flox}$ allele. In *Crim1*$^{flox/flox}$*;Pdgfb-iCreER* and *Crim1*$^{flox/+}$*;Pdgfb-iCreER* mice injected with tamoxifen, a 'deletion' band showing recombination of the genomic DNA can also be detected using the tail tip genomic DNA. Lane1: *Crim1*$^{flox/+}$. Lane2: *Crim1*$^{flox/+}$*;Pdgfb-iCreER*. Lane3: *Crim1*$^{flox/flox}$. Lane4: *Crim1*$^{flox/flox}$*;Pdgfb-iCreER*. *flox: Crim1*$^{flox}$; WT: wild type; *iCre*: *Pdgfb-iCreER*; *Δflox*: *Crim1*$^{(EC)\Delta flox}$; bp: basepairs. (C) Tamoxifen injection plan of experiments in this paper. In most experiments, tamoxifen was injected daily to pups from date-of-birth (P1) to the day of analysis (P5, P6, P7). For experiment shown in Fig. S2, tamoxifen was injected starting from P7. (D) Specificity and efficiency of *Pdgfb-iCreER* line upon injection of tamoxifen. Cre activity was visualized by labeling of X-Gal in P5 retina preps from injected *Pdgfb-iCreER;ROSA26R* pups. (E) Detection of DNA recombination of targeted *Crim1*$^{flox}$ allele in tamoxifen injected, *Pdgfb-iCreER* positive pups

Figure S2

A




B

| Stage | *Crim1*$^{\Delta flox/\Delta flox}$ embryos with anomalous phenotypes (%) | | | | | Total *Crim1*$^{\Delta flox/\Delta flox}$ |
|---|---|---|---|---|---|---|
| | eye hypoplasia | syndactyly | hemorrhage or hematoma | peridermal blebbing | wide-spread edema | |
| **E10.5** | 0 | ND | 33.33 | 0 | 0 | 3 |
| **E11.5** | 0 | ND | 0 | 100 | 0 | 2 |
| **E12.0** | 0 | ND | 0 | 100 | 0 | 6 |
| **E12.5** | 0 | 77.78 | 55.56 | 88.89 | 0 | 9 |
| **E13.5** | 87.50 | 75.00 | 50.00 | 87.50 | 12.50 | 8 |
| **E14.5** | 100 | 93.75 | 75.00 | 93.75 | 6.25 | 16 |
| **E15.5** | 100 | 100 | 62.50 | 75.00 | 0 | 8 |
| **E17.5** | 100 | 100 | 60.00 | 80.00 | 0 | 5 |

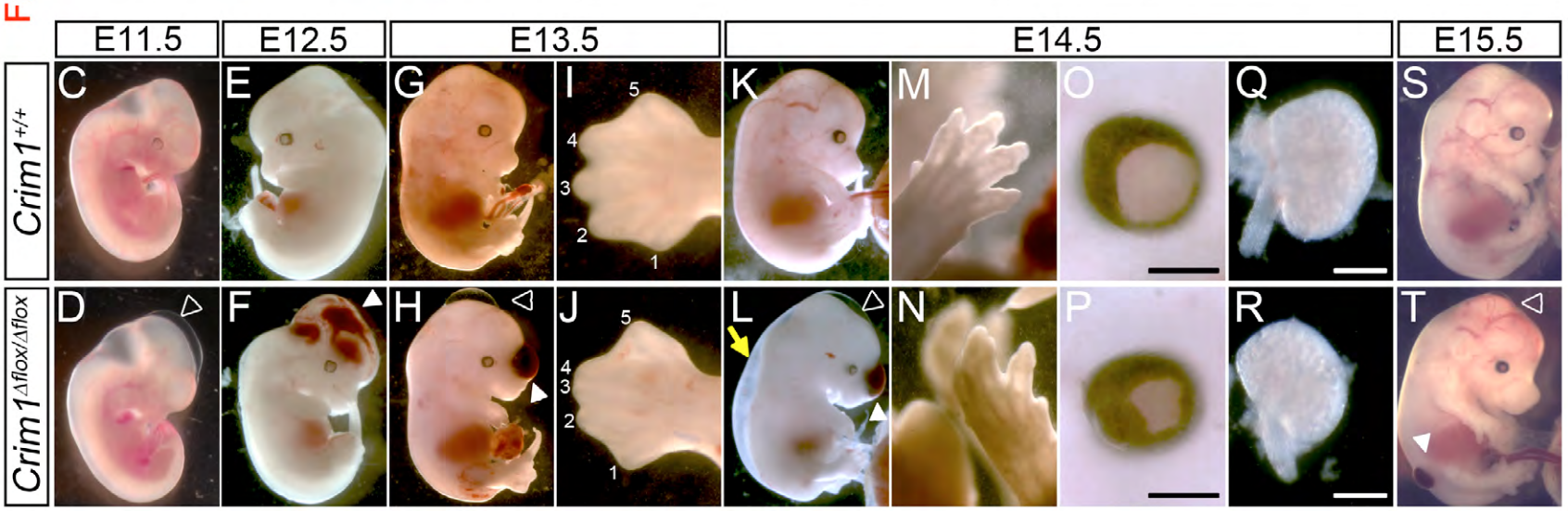



**Fig. S2. Germ line Crim1 null mice exhibited multiple developmental defects.** (A) Percentage of different genotypes in embryonic litters from intercrosses between *Crim1*$^{\Delta flox/+}$ mice at different stages. Numbers in the bars represent total embryos collected. (B) Frequency of anomalous phenotypes observed in *Crim1*$^{\Delta flox/\Delta flox}$ embryos collected at different stages. ND: not (old enough) to define a defect. (C-T) Representative images showing developmental defects in *Crim1*$^{\Delta flox/\Delta flox}$ embryos. Stages and phenotypes are indicated. Hollow arrowheads: peridermal blebbing; filled arrowheads: hemorrhage or hematoma; yellow arrow: widespread edema. (I, J, M, N) Syndactyly in germ line Crim1 null mice often happen between digits 3 and 4. (O-P) Eye hypoplasia. (Q-R) Kidneys were mildly smaller in Crim1 null mice. Scale bars in (O-R): 500μm.

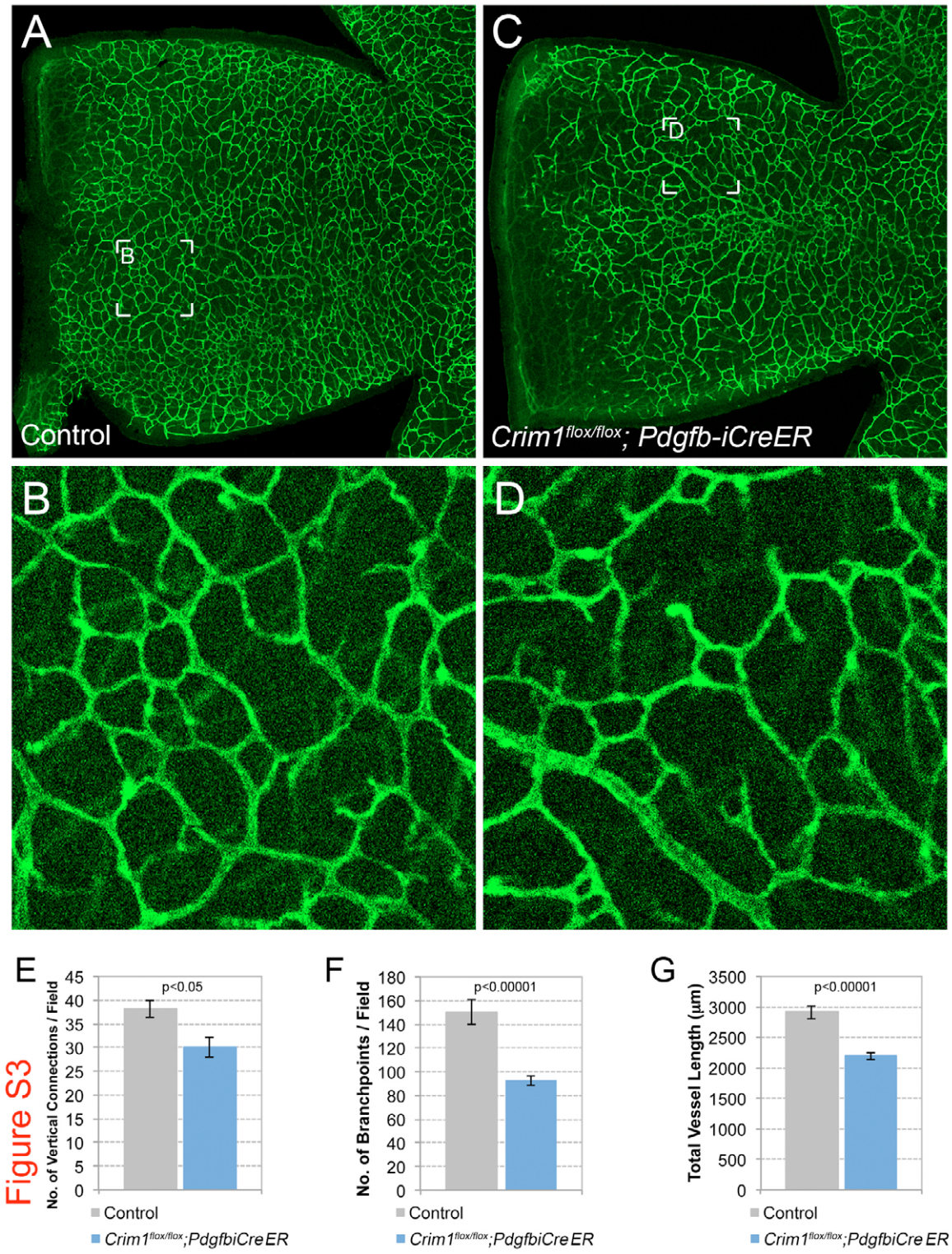



**Fig. S3. Phenotype of deep layer retinal vasculature of Crim1 VEC conditional mutant mice.** (A-D) Flat-Mounted P10 retinas labeled with isolectin IB4 showing deepest layer retinal vasculature (which is between out-plexus-layer and photo receptor layer) showed delayed vascularization of the retina. Tamoxifen was injected starting from P7 when vessel just started to sprout into the deep layer. (E-G) Quantitations of vertical sprouts, branchpoints in deep layer vasculature and vessel length in deep layer vasculature. Error bars represent s.e.m.

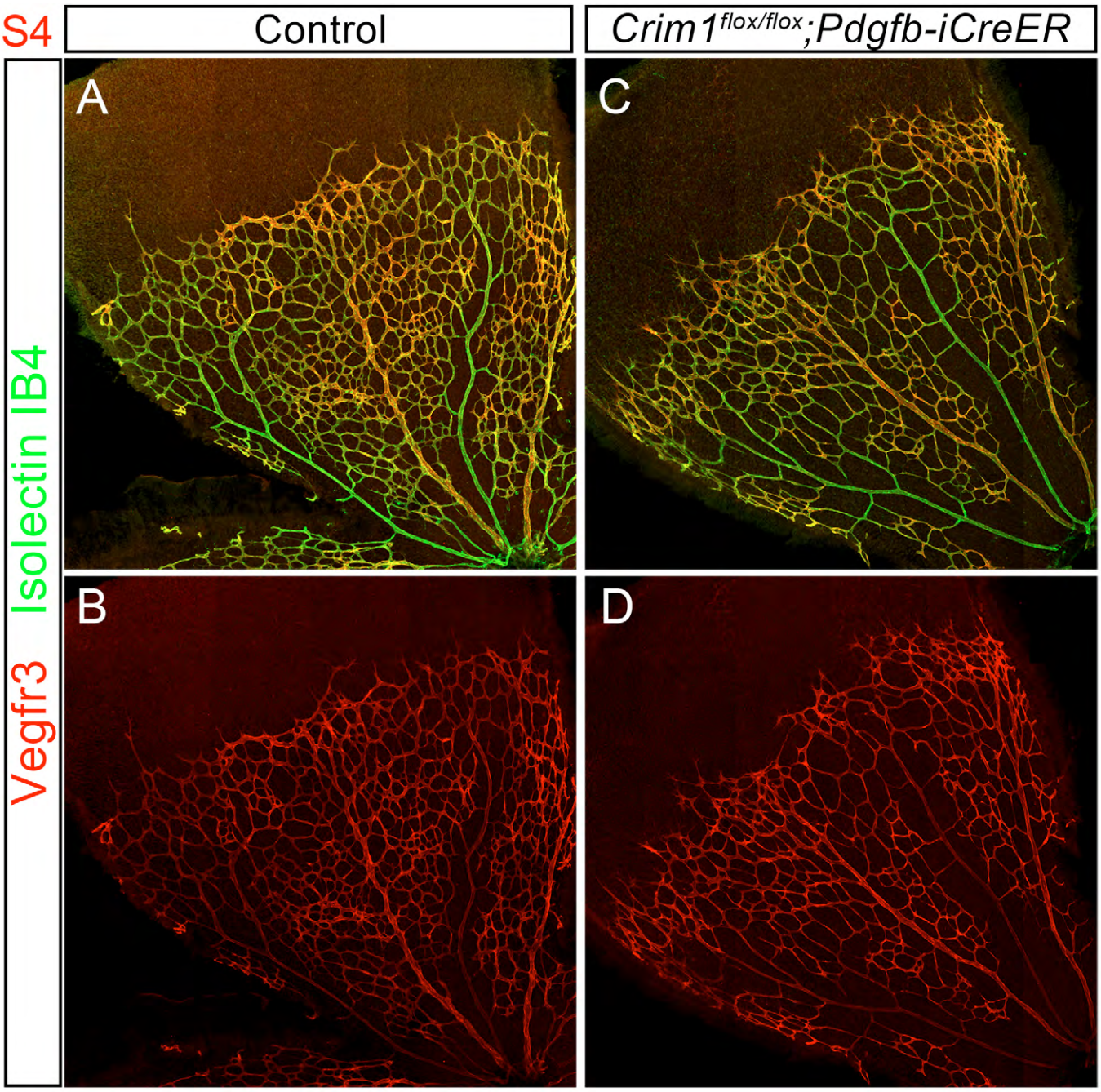



**Fig. S4. Vegfr3 expression was not changed in Crim1 VEC conditional mutants.** (A-D) Flat-mounted P6 mice retina preps labeled with Isolectin IB4 and Vegfr3 antibodies.

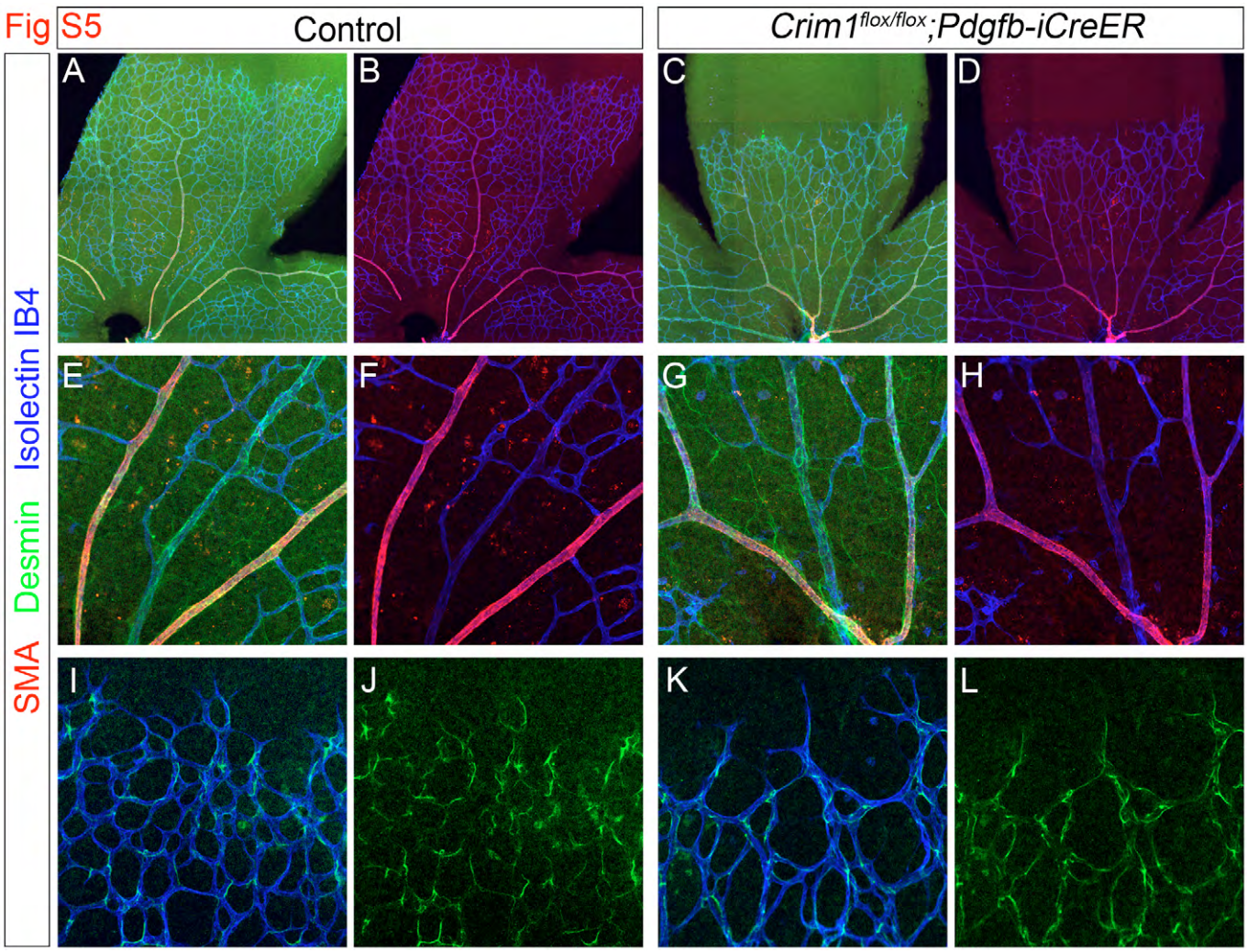



**Fig. S5. Mural cell recruitment in Crim1 endothelial cell conditional mutant mice was not affected.** (A-L) Flat-mounted P7 mice retina preps labeled with Isolectin IB4, desmin antibody (pericyte marker) and Smooth-muscle-actin antibody (smooth muscle cells marker).

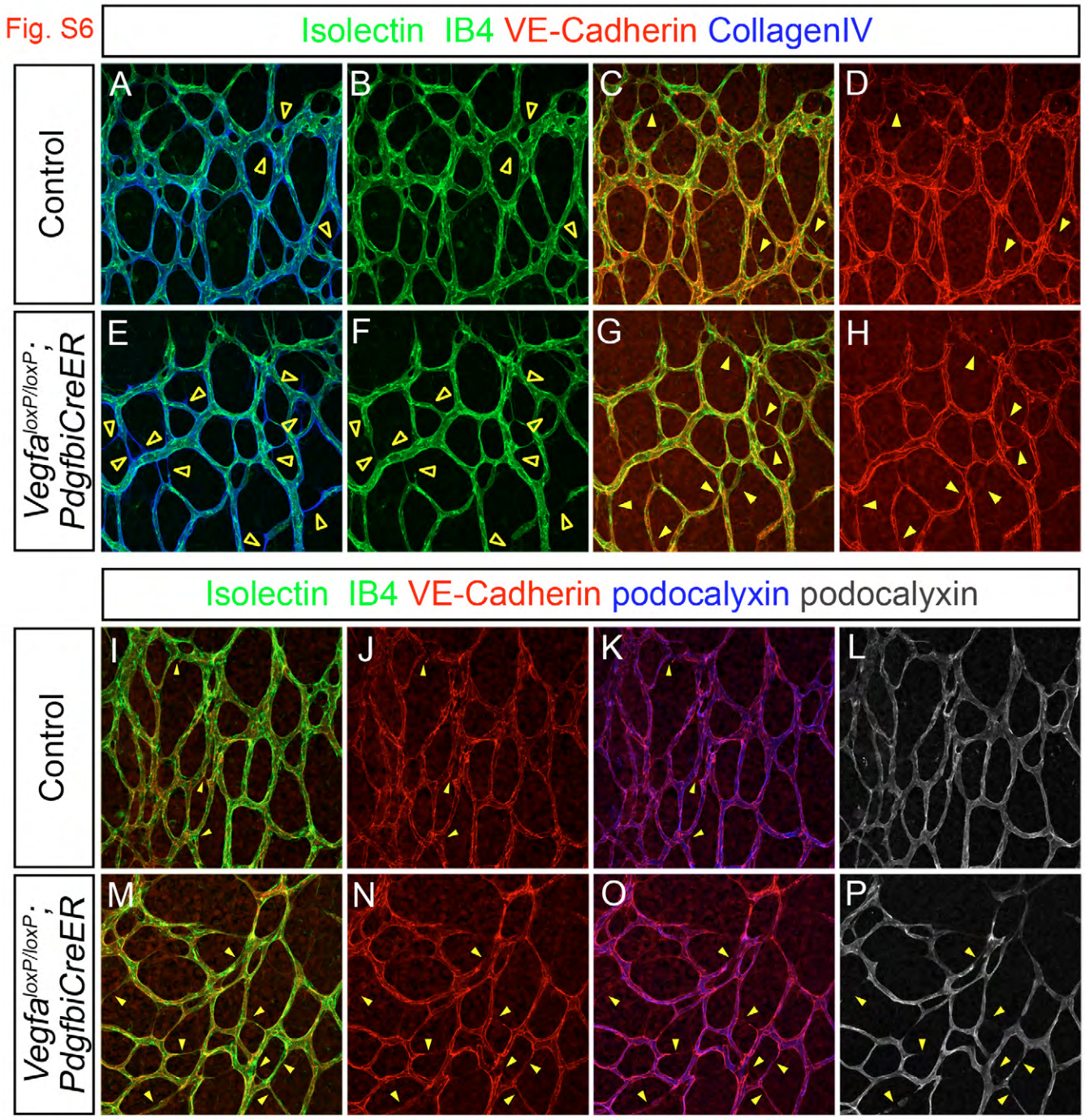



**Fig. S6. Vegfa VEC conditional mutant mice exhibited similar precocious regression phenotypes as Crim1 VEC conditional mutant mice.** (A-H) Isolectin IB4, VE-Cadherin and Collagen IV labeling of angiogenic front vasculature in control (A-D) and *Vegfa*$^{loxP/loxP}$*;Pdgfb-iCreER* (E-H) littermates. Hollow arrowheads in (A,B,E,F) point to vessel 'ghosts', indicating vessel regression and filled arrowheads in (C,D,G,H) point to VE-Cadherin labeling discontinuous regions. (I-P) Isolectin IB4, VE-Cadherin and podocalyxin labeling of angiogenic front vasculature in control (I-L) and *Vegfa*$^{loxP/loxP}$*;Pdgfb-iCreER* (M-P) littermates. Filled arrowheads point to VE-Cadherin-labeling discontinuous regions where vessels also lack luminal marker labeling.

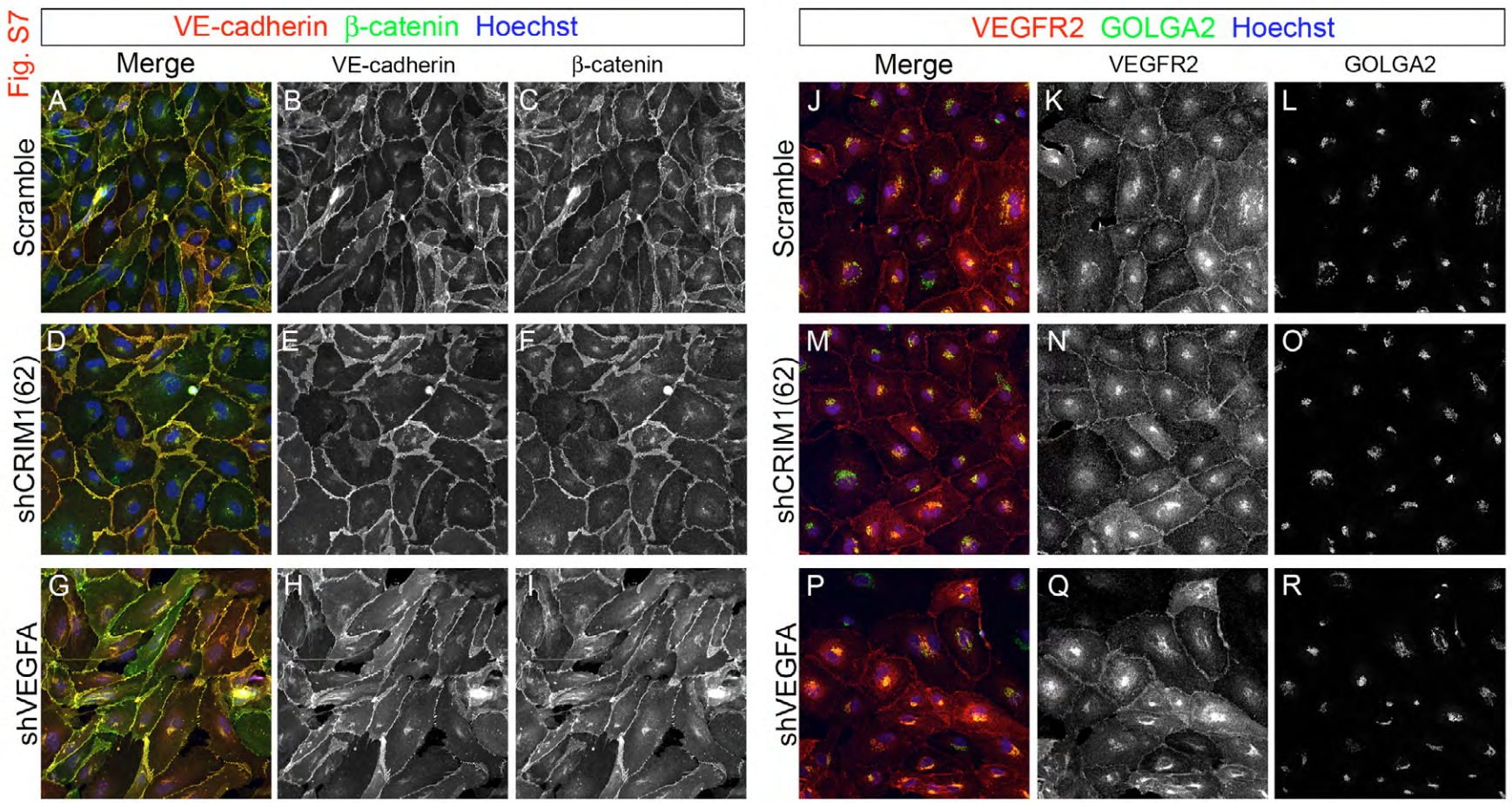



**Fig. S7. Adhesion junctions and VEGFR2 level and distribution were not changed when CRIM1 and VEGFA were knocked down in cultured endothelial cells.** (A-I) VE-CADHERIN and β-CATENIN labeling with Hoechst staining in control HUVECs or HUVECs expressing shRNAs targeting CRIM1 and VEGFA. (J-R) VEGFR2 and GOLGA2 labeling with Hoechst staining in control HUVECs or HUVECs with CRIM1 and VEGFA knock-down. GOLGA2 antibody was used to label Golgi apparatus.

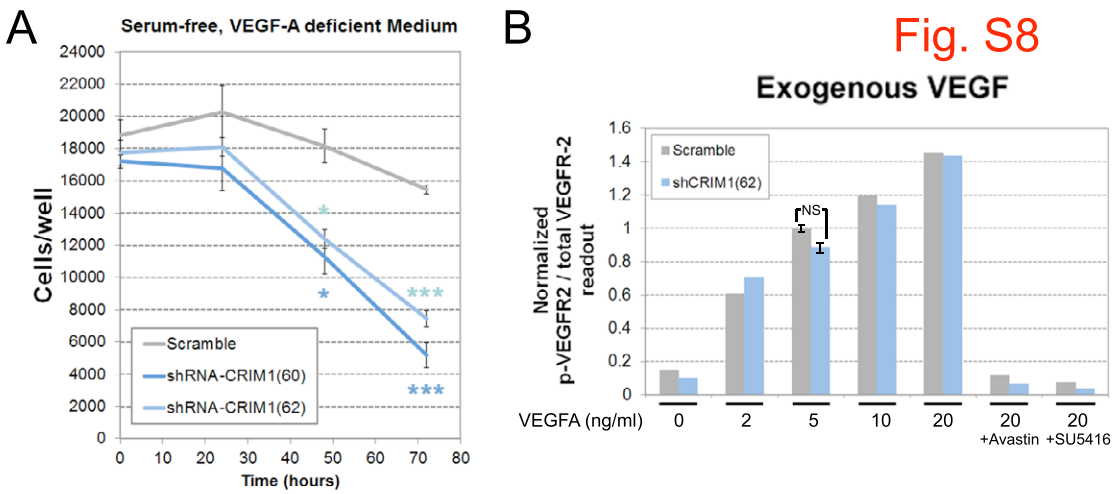



**Fig. S8. Crim1 was required for cultured endothelial cell survival.** (A) Growth curve of control and CRIM1 knockdown HUVECs cultured in serum-free, VEGFA depleted medium. 20,000 cells were plated into each well of fibronectin coated 96-well plates at time zero. (B) VEGFR2 phosphorylation in response to different concentration of exogenous VEGFA stimulation. HUVECs infected with scramble shRNA or shRNA targeting CRIM1 were starved for 24 hours and stimulated with different concentration of recombinant VEGFA for 5 minutes. Cell lysates were applied to phospho-VEGFR2 and total VEGFR2 ELISAs and the ratio of the two readouts calculated. In one set of experiments, conditional medium with 20 ng/ml VEGFA added was incubated with 10 μg/ml Avastin for 2 hours before applied to the cells. In another set of experiments, cells was incubated with 0.5 μM SU5416 for 1 hour before stimulated with conditional medium containing 20 ng/ml VEGFA with same concentration of SU5416. Error bars represent s.e.m of three experiments. *: $P<0.05$; ***: $P<0.005$.